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Bacteria of the Soil in Their Relation to Agriculture.

BY

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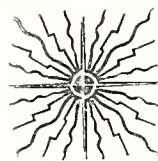


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PREFACE.

Harrisburg, Pa., August 1, 1902.

The following bulletin, No. 98, by Prof. Frederick D. Chester, Bacteriologist and Mycologist for the Delaware College Agricultural Experiment Station, upon "Bacteria of the Soil in Their Relation to Agriculture," is the discussion, by a specialist, of the phenomena that occur in the decomposition of animal, vegetable and mineral matter in the soil.

The bulletin deals with questions hitherto but little understood, and yet of vital importance to agriculture. The topic is essentially one of soil fertility, and how to secure it in the most economical manner.

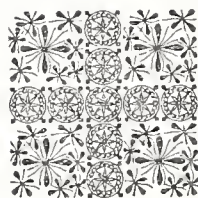
Prof. Chester describes the changes that occur in soil ingredients, and indicates the causes or agents which produce these changes, and the methods to be employed to multiply them.

Through the careful study of microscopic organisms, the most skilled scientists to-day, are reaching the true cause of disease in human beings, and other animals, and discovering remedies that are effective in preventing or controlling these diseases. By the same means, agriculture is being benefited in the study of the characteristics of the low orders of organic life, that are such potent factors in providing fertility for the soil, and in retarding or assisting, in the growth of the farmers' crops.

The facts shown in this bulletin, make more clear than ever, the truth, that the future advancement of agriculture, must chiefly be, through the assistance of science, and farming people must, if they are to succeed, become acquainted with the discoveries and conclusions of science, as they apply to their profession.

This bulletin is not only worthy of attentive study, by the individual who wishes to know the reason why he should pursue one method in his agricultural practice rather than another, but also, by the scientific man who wishes to become acquainted with the latest discoveries in bacteriology.

JOHN HAMILTON,
Secretary of Agriculture.



LETTER OF TRANSMITTAL.

Delaware College Agricultural Experiment Station,
Newark, Del., June 30, 1902.

Hon. John Hamilton, *Secretary of Agriculture, Harrisburg, Pa.:*

Dear Sir: In accordance with your request I herewith submit a bulletin on Soil Bacteriology. The subject is one which is marked by the charm of youth, and like all new subjects is attracting more or less attention. The present bulletin is a brief summary of the present status of this branch of agricultural science, and may serve as a basis for more extended reading, provisions for which are met in the appended bibliography.

Yours very truly,
FREDERICK D. CHESTER,
Mycologist.



BACTERIA OF THE SOIL IN THEIR RELATION TO AGRICULTURE.

BY FREDERICK D. CHESTER, *Bacteriologist, Delaware Agricultural Experiment Station.*

The bacteria of the soil bear a most important relation to the nutrition of plants. If a soil be heated to a temperature sufficient to destroy its bacterial life, the growth of plants will be maintained therein only up to the point of the exhaustion of its easily soluble and assimilable plant food, at the end of which time they will die of starvation. The reason for this is that new plant food can no longer be elaborated since the agents concerned in the latter process are wanting. Should this condition of sterility of the soil continue it can no longer produce crops, and were this condition universal the world would become a barren waste.

In every soil a series of complete chemical changes are taking place, due to the activities of soil organisms. These changes involve the digestion of crude plant food whereby an otherwise useless constituent of the soil is put into such a state that it can be absorbed by the plant. Digestion, therefore, implies the rendering soluble of an otherwise insoluble substance.

Nutrition whether applied to animals or plants implies three distinct processes; digestion, absorption and assimilation. Digestion is the rendering soluble; absorption is the taking up of the soluble products, while assimilation is the elaboration of new tissues from the absorbed products.

Substances to be absorbed must be so changed that they will dissolve in the fluids of the organisms, which in the case of an animal, is the blood or lymph, and of the plant, its juices.

Starch taken as food is insoluble in the fluids of the body; it therefore cannot be absorbed until it is converted into a soluble sugar. A morsel of lean meat is insoluble, however fine its state of division, hence before it can be absorbed it must be converted during digestion into a soluble pepton.

What is true of the crude elements of animal food is equally true of the crude plant food of the soil. Thus the granule of mineral matter, the bit of bone in a fertilizer, the shred of dried blood or other animal matter, the top and root of the clover turned under—

all these and many other forms of crude plant food are in themselves of no use to the plant until the elements therein are put into such a shape as to be taken up into the juices of the plant through the absorbing rootlets. Furthermore, as we have intimated, this work of digesting the crude plant food of the soil is continually being carried on by myriads of microscopic organisms present in every normal soil. Through their agency nourishment is gradually and continually being supplied to growing crops as rapidly as their needs demand, and there results a beautiful and wonderful relationship and balance between the life of the highest and lowest of the plant creation. The one is dependent upon the other, and independently neither can normally exist.

Such is the general relationship existing between soil micro-organisms and plant growth.

We are thus led to understand the importance of the study of Soil Bacteriology to general agriculture. The more detailed exposition of the subject, together with the relation of its principles to practice, will be outlined in the pages which follow.

I. THE ELEMENTS AND SOURCES OF PLANT FOOD.

Ninety-three to ninety-six per cent. of the dry weight of agricultural plants is organic matter, and is composed mainly of the four elements: carbon, hydrogen, oxygen and nitrogen. The remainder is inorganic or mineral matter which is recovered for the most part in the ash when the plant is burned.

The elements found in the organic portion occur in approximate proportions as follows: carbon 45 per cent., oxygen 49 per cent. hydrogen 6 per cent. Besides these, nitrogen may exist in amounts varying from 0.5 to 1.0 per cent. of the whole.

The green parts of all plants, but particularly the leaves, inhale and exhale atmospheric air. In the latter is ordinarily contained about four parts of carbon-dioxide for every 10,000 parts of air. Carbon-dioxide is composed of the elements carbon and oxygen in the proportion of one part of the former to two of the latter. It is this compound which furnishes to the plant all of the carbon and a portion of the oxygen.

The roots absorb water, and conduct it to the stem, whence it is carried to the leaves. Water contains the elements hydrogen and oxygen in the proportion of two parts of the former to one of the latter. Water furnishes all of the hydrogen and a portion of the oxygen. In other words, the two compounds, carbon dioxide and water, are brought together in the leaves and a chemical reaction

between the two takes place, under the action of sunlight, by which these elements are combined in such a way as to produce starch.

Starch therefore accumulates in the leaf as a result of this process known as *assimilation*.

The various changes which the starch undergoes, and the manner in which it contributes to the nutrition of the plant, is a matter beyond the limits of our subject. But suffice it to say that 98 per cent. of the organic portion of the plant is manufactured by the process here indicated, so that it may be said that in the main the plant gets its food from the air and from pure water. But these elements alone will not suffice to maintain plant life; in fact no plant can grow without that vital substance within its cells known as protoplasm.

Plants grow by a multiplication of their cells, and cells empty of protoplasm are dead.

Protoplasm, besides containing the elements, carbon, hydrogen and oxygen, also contains about 16 per cent. of nitrogen. Most agricultural plants also contain in their dry water-free state from one-half to two per cent. of nitrogen in the form of proteids.

Plants obtain their nitrogen mainly from the soil, and so important is this element to their growth that a soil may be said to be rich or poor as its contents is high or low in nitrogen. In fact the problem of agriculture to-day is to supply to the soil an abundant store of this essential element.

The nitrogen of the soil is, in the main, stored away in its humus content, hence soils rich in humus are also rich in nitrogen. Thus it is nitrogen which the agriculturist seeks when he migrates to the prairie loams rich in humus, or when he reclaims the forest to possess a virgin soil. In fact it is the nitrogen problem with which the soil bacteriologist is more concerned than with any other, and its importance and bearings will be made more apparent as we proceed.

As has been intimated, from four to seven per cent. of the dry weight of the plant is composed of inorganic or mineral matter. In this portion we recognize, as most important, potash, soda, magnesia, lime, iron, phosphoric acid, sulphuric acid, chlorine and silica.

These occur usually in abundance in all soils, although not always in an available form; in fact many of them exist in an insoluble state and need first to be digested or rendered soluble before they can be absorbed by the plant.

Soil bacteriology is partly concerned with those processes in the soil by which stores of mineral food are unlocked to growing crops. But to understand these processes in full it will be necessary to consider for a moment the question of the origin of soils, and thus trace each step in the operation.

II. SOILS, THEIR NATURE AND ORIGIN.

Rocks form the solid crust of the earth. These when brought under the influence of the atmosphere, frost and percolating waters, etc., are broken and disintegrated, forming a layer of loose materials constituting the soil. The nature of these disintegrations and their resulting products vary with each mineral, and hence the character of the soil is largely dependent upon the mineral composition of the underlying rocks.

Let us take as an example a region underlaid by some rock of the granite family, such as is found under a considerable portion of southeastern Pennsylvania. Such rock will contain the following minerals: quartz, orthoclase feldspar, plagioclase feldspar, biotite, hornblende, and accessory apatite and magnetite. The composition of these several minerals will be represented in the following table:

Table I.

Name of Mineral.	Chemical Composition.							
Quartz,	Silica.							
Orthoclase,	do.	Alumina.	Potash.	Lime.	Soda.	Magnesia.	Iron	Phosphoric
Plagioclase,	do.	do.	do.	do.		do.		acid.
Biotite,	do.	do.						
Hornblende,	do.	do.						
Apatite,								
Magnetite,							do.	

From the table it is seen that in the granite rock under consideration most of the mineral elements necessary to plant growth exist, hence a soil formed from its decay will contain the basis of fertility.

The process by which this rock becomes converted into soil is something as follows: The orthoclase, the plagioclase, the biotite and the hornblende in the above list are compounds of silica and alumina, various alkalies and earthy materials as potash, soda, magnesia, lime and iron. These latter compounds are slowly dissolved out of their respective minerals by surface waters, rain and atmospheric moisture, more or less charged with carbonic, nitrous, nitric and various organic acids until there is left behind only the silica and alumina, which combined with water form clay. The hard minerals just mentioned, bound together into a rocky mass are thus converted into a soft plastic material. The quartz on the other hand, remains undecomposed, but its grains are set free by the disintegration of the other minerals, and there results more or less sand, which, mixed with the clay, tends to loosen the latter and give it the character of an arable soil. It is not to be understood that all of the above minerals undergo dissolution uniformly. The feldspars begin to dis-

integrate first, the hornblende next, while the biotite mica remains for a long time unaffected. Thus there results a clay, the final product of the disintegration, mixed with quartz particles, or sand, together with fragments of undecomposed rock of greater or less size, giving the soil its open, porous or even stony character, so common in regions underlaid by the ancient crystalline rocks.

Besides the direct chemical actions already enumerated, other factors in soil formation of a physical nature might be mentioned. These are the expansion and contraction of rock masses; frost and freezing water; plant roots forcing their way into rocky crevices; beating and scouring rain; all tending to disintegrate the rocky covering of the earth and to open it more thoroughly to the subtle action of meteoric waters.

Another type of soils are those formed from the disintegration of limestones. Limestones are impure mixtures of carbonate of lime with various proportions of sand and clay. In the disintegration the great bulk of the carbonate of lime is leached out, and the insoluble sand and clay are left as the final product. Thus a limestone composed of 75 per cent. of carbonate of lime, may when converted into soil contain only a trace of the original carbonate. This residual soil is however more or less rich in the mineral elements of plant food while the as yet undecomposed particles in the residual sand, by continued disintegration, add new food materials to growing plants.

Sandstones undergo disintegration by the solution in meteoric waters of the materials which bind together individual grains. In this way the component sand particles are loosened, together with clay, which is generally an important constituent of most sandstones.

Whatever may be the character of the rock or of its contained minerals the process is the same, i. e., the dissolving out by means of percolating waters of the elements of plant food contained within the minerals. These percolating waters are furthermore made active solvents in the disintegration of rock through the acid products which they contain, which in turn are produced by the decay of organic matter through the agency of micro-organisms. Of the acid products the most active in this regard is carbon dioxide, which is the final product of the decomposition by bacteria of organic matter.

The chemical union between the carbon dioxide in percolating waters and the potash soda lime and magnesia in the minerals, results in the formation of carbonates and bi-carbonates of these bases, which being soluble, are in a large measure carried away in solution

so that the residual soil contains but a certain proportion of these original stores of agricultural wealth. This loss of mineral plant food is illustrated in the following table, in which in the first column are given the percentages of lime magnesia potash and soda in an original gneiss rock, and in the second column the quantities of the same present in the residual soil.

Lime (Ca O),	4.44	Trace
Magnesia (Mg O),	1.06	0.40
Potash (K ₂ O),	4.25	1.10
Soda (Na O),	2.42	0.22

The question might here be asked why are not all of these elements of plant food entirely leached from the soil, and in what form are these residual materials held. In most soils a portion of them are locked up in the form of undecomposed mineral particles and fragments of rock, and it is the continued decomposition of these latter which furnish fresh stores of available plant food.

Another important chemical process going on in the soil is the formation of so called zeolitic compounds. As the alkalies, such as soda and potash, are dissolved out of the minerals by carbonated waters the carbonates thus formed possess a certain solvent action upon silica. This dissolved or gelatinous silica combines with the alkalies, resulting in the formation of zeolites. These secondary zeolites thus fix as it were the alkalies, notably potash, which might otherwise be leached from the soil. Furthermore, the especial affinity which potash has for zeolites fixes this, the most important of mineral nutriments, above all others. Thus if a zeolite be composed of silica and soda or of silica and lime, the potash in preference will enter into combination with the silica and the less valuable soda or lime will be set free.

Zeolites differ from the more insoluble silicates found in rock-forming minerals in the fact that they are readily decomposed by acid soil waters, thus setting free to plants their valuable nutrients.

III. THE SIGNIFICANCE OF SOIL BACTERIA.

Active and Potential Fertility of the Soil.

Since the different chemical changes taking place in soils, by which plant food is elaborated and rendered available, are in large measures the result of bacterial action, it is assumed that the larger their numbers, up to certain limits, the greater must be the rate of elaboration of plant food.

This is instanced by the fact that soils which are under active fertilization and cultivation, and which in the popular sense are con-

sidered fertile, are relatively high in bacteria as compared with those in the opposite condition.

In this we must distinguish between active and potential fertility.

A soil is actively fertile when plant food is being elaborated therein at a greater rate than required by the maximum demand of growing crops.

Such soils not merely contain an abundance of crude plant food, but the latter is being actively digested.

Such soils are, furthermore, always high in bacteria, showing that the latter are functioning vigorously under conditions most favorable to them.

A soil is potentially fertile when it is rich in plant food, but owing to unfavorable conditions or environment the soil bacteria are dormant, and thus either cease to digest plant food or do it so inactive as to fail to keep up with the demand of growing crops.

Thus forest and woodland soil are rich in humus and other crude plant food, but owing to their usually acid condition, as well as to their compacted state, the bacteria therein are able to develop but slowly, and but little available plant food is elaborated. Such soils are low in bacteria; but let this virgin forest soil be brought under active cultivation, especially if its acidity be corrected at the same time by means of a liberal dressing of lime, conditions favorable to bacterial life are at once created, the number of bacteria rises, and an actively fertile soil is the consequence.

Old pasture lands and permanent meadows possess potential rather than active fertility. In such soils the number of bacteria is relatively low, and plant food is but slowly digested. But such lands are at once converted into an actively fertile condition when brought under cultivation or when other means to stimulate bacterial life in the soil are utilized.

It is the function of the agriculturist to understand how potential can be converted into active fertility; in other words how land rich in crude plant food can be made large producer of crops.

An average of the results of 49 analyses of typical soils of the United States showed per acre for the first eight inches of surface soil 2,600 pounds of nitrogen, 4,800 pounds of phosphoric acid and 13,460 pounds of potash. The average yield of wheat in the United States is 14 bushels per acre. Such a crop will remove 29.7 pounds of nitrogen, 9.5 pounds of phosphoric acid and 13.7 pounds of potash. Now if all of the potential nitrogen, phosphoric acid and potash could be rendered available there is present in such an average soil, in the first 8 inches, enough nitrogen to last 90, enough phosphoric acid for 500 and enough potash for 1,000 years.

This is what is meant by potential fertility, and yet such a soil

possessing this same high potential fertility, may, under certain conditions, be so actually barren of results to the farmer as to lead him to believe it absolutely devoid of plant food.

A soil at Rothamsted, England, which has been successively cropped to grain for 50 years without the addition of manure, and which consequently had become exhausted especially in available phosphoric acid, still contained a total of 2,880 pounds of phosphoric acid per acre in the first foot of surface.

Of this only 72 pounds per acre was soluble in a one per cent. citric acid solution. In other words, a soil which contained enough total phosphoric acid to support a wheat crop for 300 years, had, as a result of 50 years successive cropping, its store of available phosphoric acid so reduced as to leave a supply sufficient to last only between seven and eight years. This case is typical of thousands of others, and is illustrative of what is meant by soil exhaustion. It consists in using up original supplies of available plant food at a greater rate than they are being manufactured in the soil. Most of the older lands of the Atlantic seaboard which have been regarded as "worn-out" and exhausted are in much the same condition. Nevertheless they still contain large stores of unavailable plant food, which it only requires the application of modern agricultural practice to unlock. In other words, soils still potentially fertile must be made actively so, and since soils potentially fertile are low and those actively fertile high in bacteria, it would appear that one of the primary requisites of active fertility is to fulfil those conditions of the soil which favor the best development of bacterial life therein.

Numbers of bacteria in soils thus become an index of active fertility.

IV. METHODS OF DETERMINING THE NUMBER OF BACTERIA IN SOILS.

1. *Drawing the soil sample.* The determination of the number of bacteria in the soil of a given area involves an elaborate and careful preparation of the sample.

Studies at the Delaware Experiment Station have shown that the numbers vary considerably within rather narrow horizontal ranges, and thus to obtain an average sample, representative of an entire field, implies the collection and mixing of a large number of smaller samples. For most studies it will be sufficient to collect the first nine or twelve inches of soil, and for this a wood auger one inch in diameter is very satisfactory.

To preserve the boring intact a device such as is shown in Fig.



FIG. 1.—Apparatus for drawing soil samples.

I, is used. It consists of a circular plate of copper, six inches in diameter, in the centre of which is a circular hole, one and a fourth inches in diameter, from which rises at right angles to the plate a copper tube of the same diameter and twelve inches long. The ground where the boring is to be made is cleared of all vegetation and the copper plate set firmly and held in place by both feet. The auger is then inserted into the copper tube, which should stand vertical to the ground surface, and the auger turned until the required depth is reached, as determined by graduations on the stem of the auger. The auger is then drawn gently from the ground and into the copper tube until the core of earth is enclosed in the latter, and thus kept intact. The earth is then emptied on a sheet of clean paper.

In getting an average sample for an entire field, borings should be taken along two intersecting lines diagonally across a field at intervals of ten or twenty feet according to the size of the plot. These separate borings are emptied into a clean box until the work is finished. The collected soil is then sifted through a No. 10 sieve, reducing the lumps but discarding stones and gravel. The sifted soil is then very thoroughly mixed, and from this a sample of about two pounds is taken to the laboratory.

The latter sample is sifted through a brass sieve of a one-millimeter mesh, and any lumps or coarse particles are reduced in a mortar until the entire sample has been made to pass the meshes of the sieve. The whole is then very thoroughly mixed and from this a small sample of about twenty grams is taken. This is then sifted through a 0.5 millimeter brass sieve which has been made sterile in a bath of boiling water, and dried over the bare flame of a bunsen burner. Any lumps or coarse particles which do not readily pass the sieve are rubbed in a sterile mortar until the last portion has passed. The siftings are then thoroughly mixed, transferred to a sterile test-tube and tightly corked.

2. *Making the analysis.* In a weighed glass-stoppered weighing-bottle approximately 0.5 a gram of the sample is placed, and the exact weight determined. The soil sample is transferred to a small sterile mortar and with a small quantity of sterile water rubbed to a fine mud, after which the last trace of soil in the weighing-bottle is transferred to the mortar by washing with sterile water.

The supernatant muddy water in the mortar is then transferred to a 100 c. c. flask containing sterile water; more water is added and the residue triturated; again transferred to the flask, and the operation continued until all of the soil in a finely divided state has been washed into the flask. The latter is then filled to the 100 c. c. mark with sterile water, and the contents vigorously shaken for exactly

two minutes. One c. c. of this turbid water is then transferred to a second 100 c. c. flask, and filled to the mark with sterile water, shaken for one minute, and 1 c. c. of the dilution transferred to a tube of melted gelatin (5 c. c. in each tube), the latter gently rocked, and the contents poured into a Petri dish. The gelatin is made to solidify rapidly on a cold plate, and then placed in a cold water incubator for four days. At the end of this time the number of colonies on the plate are counted.

The result of the analyses are expressed in number of bacteria per gram of dry soil, hence it becomes necessary to know the percentage of moisture in the sample. For this a given weight of soil from the same tube is taken, and dried for three hours at 100° C., and from this the percentage of dry matter in the sample is calculated.

It is evident that in the mixing of the soil with water in the two 100 c. c. flasks there is in the 1 c. c. taken for the plate culture a one ten-thousandth dilution of the original quantity, hence the number of colonies on the gelatin plate must be multiplied by 10,000 to get the true number in the quantity of moist soil taken. To calculate the number per dry gram of soil the following formula is used:

$$N = \frac{N' D}{P W}$$

in which N equals the number of bacteria per gram of dry soil, N' the number of colonies on the gelatin plate, P the percentage of dry matter in the sample, D the dilution of the soil sample (in most cases 10,000), and W the weight of the moist soil taken for bacteriological analysis

V. THE NUMBER AND DISTRIBUTION OF SOIL BACTERIA.

In the superficial portion of ordinary cultivated soil the number of bacteria varies from several hundred thousand to several millions per gram of dry soil. The following list will show the range of variation as observed by different authors:

1. Park Montsouri, Paris (Miquel, 1879), ²	700,000
2. Sandy soil (Adametz, 1886), ³	300,000
3. Clay soil,	500,000
4. Orchard, Potsdam (Fraenkel, 1887), ⁴	31,000 to 218,000
5. Soil of grain fields (Caron, 1895), ⁵	937,000 to 1,600,000
6. Pear orchard, Del. Expt. Sta., ground under high state of cultivation (Chester, 1901), ⁶	2,200,000
7. Land in permanent grass for over 12 years, New- ark, Del. (Chester, 1901), ⁶	425,000
8. Land in grass for four years, Newark, Del. (Ches- ter, 1901), ⁶	425,000

9. Land, Newark, Del., under active cultivation during summer, now in crimson clover (Chester, 1901), ⁶	1,880,000
10. Soil from the center of a strip of woodland, Newark, Del. (Chester, 1901), ⁶	70,000
11. A family vegetable garden, Newark, Del. Rich in humus and actively cultivated, ⁶	1,816,000

The preceding table, except No. 10, represents agricultural soils. In special instances the number may rise much higher, particularly in soils in the immediate vicinity of dwellings and stables. According to Manfredi,^{2.1} the number of bacteria in the dust of the streets of Naples varied from 1,000,000 to 10,000,000 per gram, and even higher. Maggiora^{2.2} gives figures as high as 78,000,000 for the number of bacteria per gram of soil in certain inhabited spots.

Adametz in 1886,³ Fraenkel⁴ in 1887, and Caron⁵ in 1895, showed that the maximum number of bacteria were not found at the surface but at a depth of from nine to eighteen inches beneath the same.

In 1887 Fraenkel⁴ showed that at a depth of from thirty to sixty inches there was a rapid and abrupt diminution of the number of germs from 200,000 at twenty inches, to 2,000 at thirty-nine inches, while at a depth of five feet no living germs were obtained. These results are not altogether in accord with results obtained by the writer in Delaware, which show that the maximum number of bacteria occurs in the first six inches of soil, below which they diminish at a very rapid rate, until at twenty-four inches only about one-five-hundredth of the number at the surface exist. Furthermore, it was found that the highest numbers exist not at the surface, but at a depth of about four inches below the same. In the following figure 2 is shown the rapid decline in the number of bacteria in the soil as the depth increases, determined at the Delaware station.

VI. CONDITIONS AFFECTING THE NUMBER OF BACTERIA IN THE SOIL.

The observations of Maggiora in 1887 have shown (1) that the number of germs in desert and forest soil is much smaller, other things being equal, than in cultivated lands; (2) that the number is proportionate to the activity of cultivation and the strength of fertilizers used, and (3) that light sandy soils contain fewer germs than those rich in clay and especially those rich in humus.

These results are in accord with those given in the preceding table, which show (1) the very low number present in woodland soil, (2) the very high number present in soils under active cultivation

and (3) the relatively low number in soils covered with sod. The reason for these differences is apparent. Woodland soils, although rich in humus, are usually too acid for the best development of bacteria therein. Pasture lands, or lands for a long time in sod, are too compacted or imperfectly aerated. Most soil bacteria develop best in the liberal presence of atmospheric air, hence the opening up of such soils by tillage to the action of the atmosphere is essential before the best development of bacteria can take place. Pasture lands also have a tendency to become acid, a condition unfavorable to bacterial development.

In the studies at the Delaware Station, the highest numbers of bacteria were always found in soil which had been under active cultivation, especially when liberally supplied with humus, either by plowing under of green crops or by the use of stable manure. Thus in soil No. 6, of the preceding list, where the number of bacteria was 2,200,000 per gram, the latter had been enriched by repeated crops of crimson clover plowed under, accompanied by active tillage. Soil No. 11, a vegetable garden, had annual dressings of stable manure for a series of years, and had also been under constant tillage. The value of stable manure in increasing the number of bacteria in the soil has been shown by Miquel,² who found that after the application of this fertilizer the number of bacteria in the soil was increased from 700,000 to 900,000 per gram.

It may therefore be stated as a general principle that *the combined effect of high manuring and cultivation is to decidedly increase the number of bacteria in the soil, thus in turn setting free an increased quantity of available plant food.*

Soil Bacteria in Their Relation to Atmospheric Oxygen.

It has been stated that the great majority of soil bacteria develop best in the presence of atmospheric oxygen. Bacteria differ as regards their relation to this important element, and thus it has been the custom to divide them into three classes, (1) obligate aerobes, or those which do not grow except in the presence of oxygen; (2) anaerobes or those which grow only with the complete exclusion of oxygen, and (3) facultative anaerobes or those which are indifferent to the presence or absence of this gas. In recent times it has been recognized that no such sharp lines as these can be drawn; on the other hand these different classes merge into one another by indistinct stages of gradation.

A bacterium may, in a measure, show the ability to grow with the partial or complete exclusion of atmospheric oxygen, but it grows

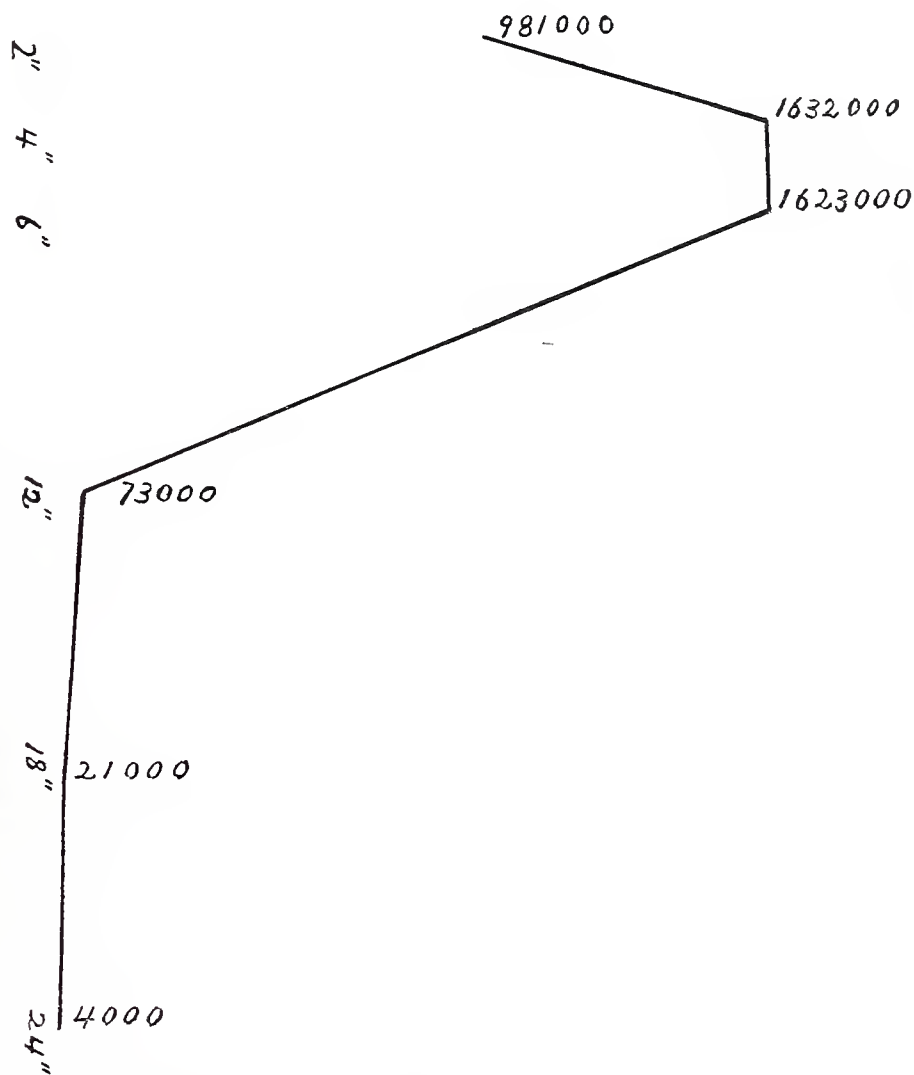


FIG. 2.—Showing number of Bacteria in different depths of soil. Experiment Station Grounds, Newark, Delaware, September 21, 1901.

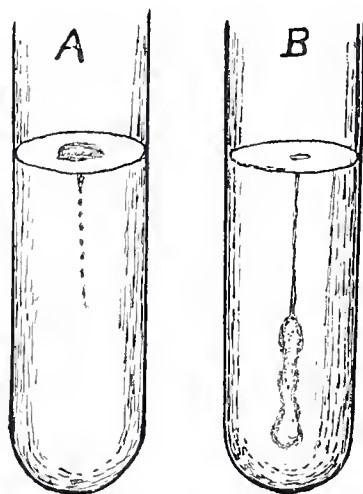


FIG. 3.—A showing aerobic growth, and B anaerobic growth of bacteria in gelatin stab cultures.

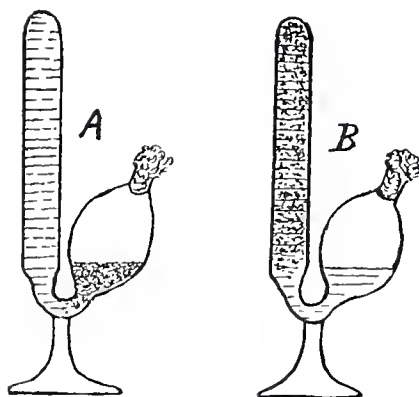


FIG. 4.—A showing aerobic, and B anaerobic growth of bacteria in glucose bouillon in fermentation tubes.

less vigorously than when air is freely admitted; in this case the tendency is towards an anaerobic habit, but such a habit may not be fixed, and it may be changed in a measure by cultural conditions under the control of the bacteriologist. That is, an organism which has a slight anaerobic habit can be made to grow more and more freely with the exclusion of atmospheric oxygen. The relation of bacteria to atmospheric oxygen may manifest itself in a number of ways.

Thus if a fine sterile platinum needle be dipped into a bacterial culture, so that its surface becomes covered with a particular germ, and if this contaminated needle be then stabbed into a tube containing solid nutrient gelatin, a medium in which bacteria grow readily, the latter are, so far as food material is concerned, free to grow at all points along the line of stab, and their development is only limited by their ability to grow in the presence or absence of atmospheric oxygen. If growth takes place as well in the depth of the medium, where air is excluded, as it does at the surface where air is abundantly present, such an organism is clearly indifferent to its atmospheric environment. If on the other hand no growth takes place in the depth of the gelatin, but only on the surface, the organism would be aerobic in habit, as shown in Fig. 3 A, or again if the growth be confined to the deeper portion of the line of inoculation with no growth at the surface, the organism would show the opposite or anaerobic habit, Fig. 3 B. Between these extremes there will in different bacteria be seen to be a wide range of variation. In the different soil bacteria so far studied there was but little tendency for them to grow in the depth of the gelatin, practically all of the growth taking place at the surface, thus showing the generally aerobic habit of the great majority of them.

Another valuable method of demonstrating the relation of an organism to atmospheric oxygen is by means of a culture in a fermentation tube, seen in Fig. 4, containing beef broth to which two per cent. by weight of grape sugar has been added. In the fermentation tube it is noticed that one end of the same is open and exposed to the air while the other is closed and excluded from the air. If bacteria are disseminated throughout the broth in the tube, they will be free to develop in either arm as they find atmospheric oxygen favorable or unfavorable to their growth. Thus if the growth is confined to the open arm of the tube the organism concerned is aerobic, Fig. 4 A, and if confined exclusively to the closed arm, Fig. 4 B, anaerobic in habit, while if an equal amount of growth takes place in both arms it is indifferent to atmospheric oxygen and is consequently facultative anaerobic in habit.

Of ten of the most common soil bacteria recently studied by the

writer, eight of them grew only in the open end of the fermentation tube, thus showing, in addition to the character of the growth in gelatin stab cultures, their distinctly aerobic habit.

In Fig. 4 A, is shown a culture in a fermentation tube to which was added 1 c. c. of a watery infusion of soil from the Delaware Station experimental grounds. In this 1 c. c. of infusion, something like 10,000 bacteria were introduced, out of the total of 1,000,000 present in the same, or one out of every hundred. It is therefore reasonable to assume that in this way at least the most important and predominating bacteria were introduced. As a result it is seen that all of the growth is confined to the open end of the tube. *Hence we may believe that at least the predominating bacteria of this soil were distinctly aerobic in habit.*

2. The Relation of Bacteria to Moisture.

One of the primary requisites of bacterial growth is the presence of moisture. If a soil becomes perfectly dry, not only do bacteria cease to multiply therein, but a large proportion of them die. It is the organic and in a slight measure, the inorganic materials in solution in the soil moisture which supply food for bacteria, hence the maintenance of soil moisture is one of the essential requisites for bacterial development. In short, those moisture conditions which are most favorable to the plant are likewise equally favorable to the bacteria of the soil. A free and uniform distribution of soil moisture is furthermore essential to a uniform distribution of bacteria, and hence to the active elaboration of plant food in all parts of the soil. It is clear that when the soil becomes dry to a considerable depth bacteria cease to develop and with it the digestion of plant food ceases; hence the maintenance of soil moisture by proper methods is important.

3. Relation of Soil Bacteria to Organic Matter and Humus in the Soil.

Inasmuch as organic matter and humus furnish food for soil bacteria it might be presumed that the greater the amount of such materials present the greater would be the number of bacteria and hence the greater the amount of plant food digested. Such is the case only within limits. If a solution be prepared containing one per cent. by weight of beef pepton, and be seeded with a soil organism capable of converting the pepton into ammonia and other decomposition

products, a vigorous growth will take place, and a relatively large amount of decomposition products will be quickly formed; but soon active growth will cease, and that before the full amount of the original pepton has been completely decomposed. In short, the organism has been either killed or its energies have been paralyzed by the products of its own growth, which in this case have been produced in relatively large amount in the more concentrated solution. If on the other hand a solution be prepared containing only the one-hundredth of one per cent. of pepton, and be seeded with the same organism, growth in the latter medium will be relatively slow, with a correspondingly slow development of ammonia; but the decomposition will continue until all of the pepton has been decomposed. Furthermore, the activity of the decomposition will be as great at the end as at the beginning of the process, showing that the vitality of the organism has not been impaired. This is doubtless because in the dilute solution the toxic products are not sufficiently concentrated to injure the life of the micro-organisms.

What is true here would be equally true of the state of concentration of the organic matter in the soil. If the latter be present in excessive quantity bacterial development will proceed for a time at an excessive rate, but soon products injurious to their best development will be produced. There is, therefore, a limit to the amount of humus which a soil should contain.

In forest and woodland soils the amount of humus in the surface layer is large. In such soils organic acids are generated in quantities too large for the best development of bacteria, and hence, as is found to be the case, the number of bacteria is low.

It has been shown that nitrification practically ceases in forest soils, due doubtless to the fact that the nitrifying bacteria, more than any other, are injured by high acidity and excessive humus.

The only condition which renders possible the addition of large stores of humus to the soil is subsequent tillage, which so stimulates bacterial growth as to lead to the destruction of organic acids, or to the production of ammonia, which neutralizes them. *Hence when a crop of clover or other legume is plowed under it is best followed by a cultivated or hoed crop.*

4. The Relation of Soil Acidity to the Number of Soil Bacteria.

It is important to know the conditions of the soil which are most favorable to the rapid development of soil bacteria. Among these nothing is so important as to maintain a proper reaction of the soil. Acid soils are infertile because soil bacteria, which are digesters of plant food, cannot grow therein. We say that lime when applied to

land, among other benefits, assists in the decomposition of organic matter. This is true only indirectly. The lime neutralizes the acidity of the soil and renders it a more favorable medium for the development of those bacteria which are the true agents in the decomposition.

In determining the number of bacteria in soils it is necessary to use a medium the reaction of which is such that the maximum number will develop. Thus from a given dilution of a soil with water an average of seventy-two colonies developed in neutral gelatin, while with the presence of 0.25 per cent. of free alkali an average of thirty-four colonies developed, and with .07 per cent. of free hydrochloric acid an average of only one colony developed. Increasing quantities of citric acid added to the medium had the same retarding action, showing that with a distinctly acid condition of the medium but few soil bacteria would grow.

Of the different species of bacteria isolated by the writer from soils, none grew in a medium containing one-tenth of one per cent. of free hydrochloric acid, and either not at all or only feebly in one containing one-half of this acidity. All, however, grew in neutral media or in those feebly alkaline. A marked excess of caustic alkalies in the medium, approaching .02 to .03 per cent., had a retarding action on the growth of the bacteria, but where the less caustic bases like lime were added a considerable excess proved favorable.

The valuable results from lime added to neutral gelatin media is shown in the following table, in which is given the number of colonies developing in media containing different amounts of milk of lime, seeded with the same quantity of a one-ten-thousandths dilution of a soil infusion:

Table II.

Calcium Hydrate (Ca (OH) ₂) Present in Medium.		Number of colonies which developed in the medium.
.44 grams per 100 c. c.,	57
.08 grams per 100 c. c.,	75
.12 grams per 100 c. c.,	82
.16 grams per 100 c. c.,	91

The valuable results from the use of lime seem to depend partly on the fact that it stimulates the development of soil bacteria. This was shown in certain pot experiments conducted by the writer.

Pots were filled with soil to which was added an equal quantity of clean gray stream sand.

To pots one and two nothing was added.

To pots three and four was added lime at the rate of 1,000 lbs. per acre.

To pots five and six was added lime at the rate of 2,000 lbs. per acre.

To pots seven and eight was added lime at the rate of 4,000 lbs. per acre.

The number of bacteria per dry gram of soil in each pot was determined at the beginning of the experiment, and again seven weeks later.

The results are shown in the following table:

Table III.

Pot. No.	Number of Bacteria Per Gram of Dry Soil.	
	At beginning of the experiment.	Seven weeks later.
1 Nothing,	441,000	440,000
2 Nothing,	567,000	395,000
3 1,000 lbs. lime,	734,000	1,026,000
4 1,000 lbs. lime,	703,000	2,076,000
5 2,000 lbs. lime,	908,000	1,325,000
6 2,000 lbs. lime,	406,000	1,319,000
7 4,000 lbs. lime,	457,000	4,481,000
8 4,000 lbs. lime,	504,000	6,662,000

The preceding experiment has been repeated with the same result, sufficient to demonstrate the value of lime, at least in the type of soils under consideration, in increasing the number of bacteria therein.

VII. THE CHEMICAL CHANGES PRODUCED BY BACTERIA IN THE SOIL.

THE ELABORATION OF PLANT FOOD.

The processes going on in the soil by which plant food in its crude state is prepared for the use of the growing crop are two, (1) the decay of organic matter and (2) the disintegration and dissolution of mineral matter. They will be accordingly considered in turn.

1. The Decay of Organic Matter.

Organic matter, whether of animal or vegetable origin, when freshly incorporated with the soil, undergoes a partial and incomplete process of decay resulting in the production of a dark material known as humus.

The amount of humus in soils may vary from one per cent. in the soils of the arid region of the West to as high as five per cent. in black prairie loams.

The original supply of humus in virgin soils becomes a constant source of plant food through its slow but constant decay. Long continued cropping and tillage produces in time, however, a "burning-out," which means that the humus content of the soil is being gradually reduced. Thus according to Snyder,⁸ a virgin soil with four per cent. of humus will, after twenty years of grain cropping, show a reduction to 2.5 per cent. of the same material.

2. Forms of Organic Matter in the Soil.

Organic matter becomes incorporated with the soil largely in the form of vegetable materials such as fallen leaves, sticks, seeds, straw, stubble, sod, the roots of various plants, green crops turned under, etc.

Such vegetable matter of whatever kind is composed of a framework of cells constituting its woody portion. The material constituting the walls of these cells is, for want of a better term, designated as cellulose, since the latter substance in one form or another predominates. Within the cavities of the cells, partially or completely filling them, may be found certain organic substances of which the most important are: (1) proteid matter, including protoplasm, proteid granules, aleurone and gluten; (2) carbohydrates, including starch, grape sugar, cane sugar, vegetable mucilage, gums; (3) fats and oils. Besides these are small quantities of a great variety of other substances, as glucosides, tannin, alkaloids, essential oils, resins, balsams, turpentine, coloring matter, etc. Notwithstanding the great variety of plant products, the principal materials, forming practically

the entire bulk of plant structures, are included under the heads (1) cellulose, (2) proteid matter, (3) carbohydrates and (4) fats and oils. The decomposition of these several materials in the soil will be considered each in turn.

3. The Decomposition of Cellulose or Vegetable Fibre.

That straw and bits of woody fibre become soft and finally disappear as such when incorporated with the soil is a fact of common observation. Leaves and stems when mingled with earth rapidly lose their structural characteristics and become converted into a shapeless mass of mould. The log or stump under the action of biological and chemical agents decays more slowly, but eventually loses its structure and becomes converted into a brownish pulverent debris.

These changes involve the fermentation of cellulose or vegetable fibre, and are of special interest. The walls of vegetable cells are composed of matter more or less complex in character; but since cellulose in one form or another constitutes the basal portion of all cell walls, it has been common to refer to them as composed of this substance. But more accurately speaking cellulose is now understood to include a large class of plant constituents.

These latter may be grouped under two heads: (1) the celluloses and (2) the pectoses. The walls of different cells differ in the relative proportion of these two classes of bodies. Thus the walls of cells which constitute so called succulent or parenchyma tissue are relatively rich in pectoses. This is particularly marked in the flesh of fruits. Cellulose differs in its properties and ability to undergo fermentative changes. In the latter respect cotton fibres are the most resistant and the cellulose of seeds the least so, while that found in the fundamental tissue of the higher plants occupies an intermediate position. With the difference in the constitution of the cell walls of plants there results a marked divergence in their ability to undergo fermentative changes, and also a difference in the products of such fermentation. For this reason the fermentative decomposition of cellulose becomes an extremely complex phenomenon.

The dissolution of cellulose is brought about by the action of a ferment or *enzyme* known as *cytase*.

In 1886, DeBary,⁹ found in the fungus *Peziza sclerotiorum* a substance which possesses the property of causing cell walls to swell, become gelatinous, and in a measure to dissolve. Two years later H. Marshall Ward,¹⁰ found that a similar ferment was secreted by a species of *Botrytis*, commonly associated with the soft rot of a number of cultivated plants.

In his study of the latter, the author observed minute drops ex-

uding from the filaments of the fungus, and in this exuding fluid was found a ferment in concentrated form, possessing the power when coming in contact with cell walls of softening and dissolving them. It was also observed that the ferment acted differently on different portions of the cell wall, and that its action was first upon the middle portion, or what is technically known as the *middle lamella*. This was followed by a swelling of the remainder of the wall, and by the appearance of distinct stratifications, which dissolve one by one in turn. In the swelling of the cell walls the latter assume a semi-mucilaginous consistency which has the effect of softening the entire tissue. Thus plants attacked by cytase secreting fungi, such as the ones named, undergo a species of soft rot.

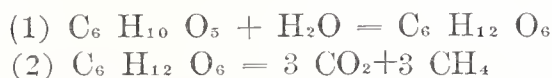
In addition to the preceding observations Kean and Arthur¹¹ have recently shown that the fungus *Rhizopus nigricans* also secretes a cellulose dissolving enzyme. This latter fungus is a common cause of a soft rot of the sweet potato, a result in accord with the properties of the fungus. It is, furthermore, probable that a large variety of fungi associated with the soft rots of fleshy fruits and roots, possess the same property of secreting cellulose enzymes.

A number of the higher toad-stools and shelf-fungi, are associated with dry rot of timber, in which process the hard wood becomes converted into a brown pulverent mass. This disintegration is affected, it is now believed, through the ability of these several fungi to produce enzymes capable of softening and in a measure at least of dissolving cellulose or woody tissue.

Besides the fungi proper, certain bacteria have been shown to possess the ability to ferment cellulose. Thus as early as 1850, Mitscherlich¹² made the observation that cellulose could become soluble by fermentation. In the fermentation of the potato for instance he found the cell walls dissolved, and associated with this change he noted the presence of a species of Bacterium. In 1875, Popff¹³ noted the relation between the degree of fermentation of cellulose and the development of certain gases, as carbon dioxide (CO_2) and marsh gas (CH_4). Later, in 1879, Van Tieghem showed by experiment that a solution of cellulose was effected through the action of a micro-organism related to *Bacillus amylobacter*. During the change, hydrogen gas was generated, also an acid, whose presence gradually hindered the fermentation process. Van Tieghem's observations that the fermentation of cellulose was due to the latter *B. amylobacter* was confirmed by Hoppe-Seyler¹⁵ in 1886.

This fermentation as originally shown by Popff, and later by Hoppe-Seyler and Schlösing, was accompanied by the vigorous evolution of carbon dioxide and marsh gas, and took place in the absence of air.

The process consisted probably in an hydration of the cellulose, and its conversion into dextrose or a related body, and the subsequent fermentation of this secondary product as shown by the following formulae:



In 1890, Von Senn¹⁶ showed that the fermentation of cellulose was not due to the action of *B. amylobacter* alone, but to its concurrent action with other organisms. In 1895, Omélianski¹⁷ announced the discovery of a bacillus capable of fermenting pure cellulose, which he obtained from slime and soil rich in vegetable matter.

In the experiments of the author filter paper or cotton, representing cellulose in its purest form, was immersed in a solution containing sulphate of ammonia, pepton and asparagin, and into this culture medium the organism was introduced. The beginning of the fermentation was shown by the liberation of gas in from 6 to 10 days. An examination of the filter paper in from 3 weeks to a month showed an advanced stage of decomposition, and in from 3½ to 5 months 79 per cent. of the cellulose had been destroyed. The products of the fermentation were found to be carbon dioxide, hydrogen, volatile organic acids and minute quantities of the higher alcohols.

Whether bacilli identical with *B. amylobacter* of Van Tieghem, or the Bacillus of Omélianski, are found in all soils is a matter yet to be determined, but it is believed that organisms with similar functions are present in abundance. Furthermore, whether these bacteria decompose cellulose through their ability to secrete enzymes has also to be determined. DeBary in referring to *B. amylobacter* says it decomposes cellulose forming dextrin and glucose, and that it does so by disengaging an enzyme.

Although the experimental proof of this is lacking it is probable that the assumption is true.

Bacillus mesentericus-vulgatus, a common soil species, has been shown by Vignal¹⁸ to secrete a cytase which dissolves the middle lamella of vegetable cells.

There is therefore every reason to believe that numerous organisms capable of fermenting cellulose exist in soil, and that they act upon cellulose, like the higher fungi, through their ability to produce cytase.

The action of cytase upon cellulose is to incite a chemical union of water with cellulose, a process known as hydrolysis, and is an action similar to that which takes place when cellulose is boiled in

dilute acids. It consists in the conversion of the cellulose into some form of sugar, which differs with the forms of cellulose acted upon. These different forms of sugar are glucose, mannose, galactose, zylene and arabinose.

The pectoses, which have been found also to be important constituents of the cell wall, are under the action of cytase converted into reducing sugars. The different forms of sugar are then acted upon by other ferments and converted into organic acids.

This explains the common tendency of soils rich in vegetable matter to become acid, unless continued cultivation stimulates bacterial growth sufficient to decompose these less readily decomposable organic acids into their final gaseous products, carbon dioxide and marsh gas.

It has already been noted that all of the constituents of cell walls do not undergo dissolution equally. Hence when vegetable fibre undergoes fermentation in the soil there remains a residue which for a longer time withstands the action of these ferments. This latter constitutes the great bulk of that heterogeneous material which is called humus. Humus is, therefore, in the main, the product of the incomplete decomposition of vegetable fibre.

4. The Fermentation of Carbohydrates.

The carbohydrates in vegetable tissues exist mainly in the form of starch and sugar. In crops ordinarily used for green manuring they constitute between 40 and 50 per cent. of the dry weight of the plant. Starch is the most abundant carbohydrate of green crops, but sugar exists in small amounts, usually a fraction of a per cent. However, in fleshy roots, fruits, and in special cases, it may run much higher, reaching a maximum in the beet and sugar-cane of 15 per cent.

Sugar exists in different vegetable tissues in three forms: as cane sugar or saccharose, grape sugar or dextrose and fruit sugar or levulose. In animal tissues and fluids the carbohydrates exist: as glycogen, a modified starch, as dextrose, and in milk as milk sugar or lactose.

Bacteria play an important role in the fermentation of carbohydrate, and those concerned in these processes are abundantly present in all soils. The great majority of bacteria when growing in media containing grape sugar, milk sugar or cane sugar produce therein greater or less quantities of organic acids accompanied in some cases by the evolution of gas.

These acids are lactic, acetic, butyric, formic, propionic, valerianic and succinic. When milk sours, lactic acid is produced at the expense of the milk sugar by certain bacteria normally present in the

fluid. Fruit juices and infusions undergo an apparently spontaneous fermentation, the sugar being converted into alcohol through the agency of the yeast plant, with the subsequent conversion of the alcohol into acetic acid.

Many bacteria are, however, capable of directly converting sugar into one or more of the organic acids, without the intervention of alcohol; in fact, it may be said that the ability to convert sugar into one or more of the organic acids is almost a universal property of bacteria, although they vary among themselves as regards kinds and quantities of acids produced.

In order to better understand the fermentation of the different carbohydrate constituents of plants, it will be best to consider them in turn.

(a) *The Conversion of Starch into Sugar.* When seeds germinate, a marked change takes place, the most notable of which is an alteration and an eventual solution of the starch granules which fill the cells. In proportion as the starch disappears there is a corresponding increase in sugar. In the preparation of malt from barley the same change takes place. The barley grains are allowed to sprout under favorable conditions of heat and moisture, during which a considerable proportion of the insoluble starch is converted into a soluble sugar. If a quantity of this malt be steeped in water, especially if the malt be macerated to a pulp, the greater portion of the sugar, a part of the soluble starch and dextrine bodies and other extractive matter pass into solution. If a portion of this extract of malt be allowed to act upon starch it will be found to possess the power of converting the starch into sugar. Furthermore, if several volumes of strong alcohol be added to a volume of the malt extract a whitish precipitate will be thrown down, which can be collected on a filter and redissolved in a small quantity of water. If now this watery solution be allowed to again act upon starch, it will be found to possess properties identical with that of the simple infusion.

Malt extract, therefore, contains a substance which is precipitated by alcohol and which has the power of converting starch into sugar. This substance is called *diastase* or *amylase*.

Diastase has an important function in relation to the nutrition of plants. Plant food exists largely in the form of starch, but which in this shape is of no use, since it is insoluble and therefore incapable of being carried in solution to growing parts; in short, the starchy food of the seed must be digested before it is available, and this digestion is effected through the agency of diastase. Similarly, starch is formed in the leaves and other green organs of the plant, but before it can be utilized as food it must be converted into sugar. To effect this change diastase is present in all leaves and organs where

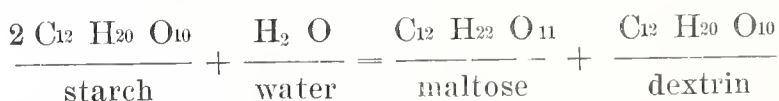
starch is being elaborated. Many trees store up during the winter reserve material in the form of starch which becomes food for unfolding buds on the advent of spring. Thus Desbarres¹⁹ found in the young wood of *Rhus elegans* 17.31 per cent. of starch during the winter and only 1.57 in the spring.

The sugar maple yields in the early spring a sweetish sap which is produced from the reserve starch accumulated in the wood during the preceding fall.

Many roots and tubers are notable for their large content of starch, which, in all biennial plants, serves as food for a second season's growth. When potatoes sprout they draw largely upon the starch of the tuber, and with the elongation of the sprouts we note a diminution of the starch and an increase of sugar. With this there is an accumulation of diastase in the tuber at the points where the sprouts originate.

In referring to the fermentation of cellulose, it was stated that it is a common function of many enzymes, of which diastase is one, to effect the hydration of certain organic substances, or their union with water.

The hydration, or conversion of starch into sugar, is a complex process, not as yet altogether understood, but the two products of the change are evidently maltose and dextrin. It can perhaps be expressed according to Musculus²⁰ by the following:



Dextrin, which is a residual product of the partial hydration of starch does not, however, remain as such, but is eventually converted into maltose.

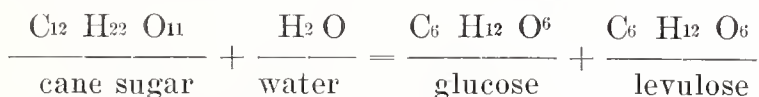
When vegetable materials are incorporated with the soil, it is not likely that bacteria play a very important role in the conversion of this contained starch into sugar. On the other hand the change is likely brought about by diastases normally present in the plants themselves, and the greater part of this transformation is effected before bacteria have time to reach the starchy materials within the cells. Thus in the decomposition of vegetable matter in the soil, much at least of the fermentation of starch is a process quite independent of the action of bacterial life. This does not indicate, however, that certain bacteria and fungi are not capable of effecting this change, in fact Fermi²¹ has shown that a considerable number of bacteria secrete diastatic enzymes, notably, *Bacillus megatherium*, *Bacillus mesentericus-vulgatus* and *Bacillus subtilis*, forms commonly present in the soil; and it is likely that a residual portion of unchanged starch may be acted upon by such organisms, though this

statement will require experimental proof. It is also known that certain common moulds, for example, *Pennicilium glaucum*, *Aspergillus niger* and *Erotium oryzae*, possess the power of converting starch into sugar.

As already mentioned, the action of diastase upon starch consists in its conversion into maltose and dextrin, but maltose has only a temporary existence in the animal and plant organism, since it is acted upon by another enzyme known as *glucose*, which further hydrolyses the maltose converting it into glucose. Thus glucose is the final product of the fermentation of starch.

Glucose is present in the digestive fluids of the human body. It has been found in corn and malt, and in several species of fungi. That it probably exists in association with diastase in plant tissues is made probable by the fact that maltose as such is unable to nourish growing cells.

(b) *The Inversion of Cane Sugar*.—When a solution of cane sugar is boiled with a dilute acid it undergoes an hydrolysis by which it is converted into glucose and levulose according to the equation:



That the same change can be effected through the agency of an enzyme has been known ever since the latter was first isolated from yeast in 1860 by Berthelot.²² This enzyme is known as *invertin*. It has also been found in the intestinal juices of man and a number of animals, and from various parts of plants as leaves, seeds, roots and floral organs.

Cane sugar is found often in considerable amounts in plant tissues, and yet as such it is of no direct use as a plant nutrient, but must first be digested or converted into glucose. Thus the beet may contain 15 per cent. of cane sugar. When, however, the latter is drawn upon for the production of flowers and seeds during the second year's growth it has been noted that its content of cane sugar gradually diminishes and glucose takes its place, the latter being traced in its ascent from the root to the developing leaves and flowers. Thus the presence of cane sugar in the plant implies the existence at the same time of invertin. The first step therefore in the fermentation of cane sugar is its conversion into glucose through the agency of its associated *invertin*.

This change like the action of diastase is also one which takes place in the soil independent, in a large measure at least, of bacterial action.

According to Fermi²¹ and Montesano the production of invertin enzymes by bacteria is uncommon, although certain prevalent soil

species, such as *Bacillus megatherium*, *Bacillus fluorescens-liquefaciens* and *Bacillus vulgaris*, are known to produce them.

The action of invertin is favored by the presence of small amounts of acid; it is therefore likely that in the acid fermentation of plant tissues and the normal presence of invertin ferments there is every condition favorable for the conversion of all the cane sugar into glucose independent of the action of bacteria or other micro-organisms.

(c) *The Fermentation of Glucose*.—From the foregoing statement it has been seen that all of the carbohydrates mentioned are eventually converted into glucose mainly through the action of enzymes. It is in this form that they are supplied to the various soil organisms. Through their agency glucose is converted into the various organic acids, into one or more of the alcohols, with or without the evolution of gas in the form of carbon dioxide and hydrogen.

The great majority of bacteria possess greater or less power of producing one or more of the organic acids from glucose, although much work has yet to be done in determining the kinds of acids produced by different species. The following table shows the products of the fermentation of glucose by a number of common bacteria:

Bacillus acidilactici—acetic and lactic acids, traces of alcohol and gas.

Bacillus aerogenes—acetic, lactic and succinic acids, alcohol, carbon dioxide and hydrogen.

Bacillus typhosus—lactic acid.

Bacillus coli—acetic, formic and lactic acids.

Bacillus prodigiosus—formic and succinic acids.

Bacillus butyricus Botkin—acetic, butyric, formic, propionic, lactic, and succinic acids; butyl and ethyl alcohol, carbon dioxide and hydrogen.

Bacillus amylozyma Perdrix—acetic and butyric acids, carbon dioxide and hydrogen.

Cholera Micospira—lactic acid.

Micrococcus pyogenes—lactic and valerianic acids.

Streptococcus pyogenes—lactic and volatile organic acids.

The organic acids produced by the fermentation of glucose tend to combine with any free base in the soil such as lime, soda, potash, and, in a measure, to decompose carbonates. But where this base is not present in sufficient quantity the free acids accumulate and the soil becomes sour. Under active cultivation, however, the acids and their salts undergo a still further fermentation whereby they are converted into carbon dioxide and marsh gas (CH_4).

Thus cultivation has a tendency to overcome acidity by stimulating the growth of those bacteria which destroy organic acids.

(d) *The Action of Oxidizing Enzymes in the Fermentation of Vegetable Matter.*

It is a common phenomenon that where grass or green hay is made into a pile the interior will begin to ferment, and with this there will be a considerable rise of temperature. A similar process goes on in the manure heap, and another when green fodder is packed in the silo.

In the silo the temperature in the center of the fermenting mass may rise as high as 150 degrees F. With this fermentation there is a considerable loss of organic matter which may vary from 4 to 40 per cent. The temperature as well as the loss of material in the silo is dependent upon the amount of air, or more properly the oxygen present, and this depends upon the looseness or density of the packing. The change is manifestly one of combustion due to the absorption of oxygen, and the products of this change are the same as those evolved in any other combustive process, i. e., carbon dioxide and water. Furthermore, the amount of carbon dioxide evolved is a measure of the degree of combustion and of the organic matter consumed, as well as of the heat produced.

Formerly it was supposed that the fermentation of silage was due to the agency of bacteria, but now it is believed to be simply an expression of the vital energies of the plant cells. All vital energy manifests itself in the production of heat; this heat is the result of oxidation, or the actual burning or destruction of a portion of the vital substance. Yeast when massed into a heap shows a rise of temperature due to its vital energies. This rise takes place only in the presence of oxygen or air, and in a vacuum no such increase of temperature occurs. The germination of seed is accompanied by a rise of temperature, and oxygen is necessary to the process.

The animal body gives off heat and the air we breathe is the draught for this ever consuming fire within, while the carbon dioxide exhaled is a measure of the rapidity of this combustion process.

In a similar manner plants evolve heat and their substance is, in a measure, oxidized or burned to supply this heat, a portion of which is converted into the vital energies of the plant.

Respiration is the breathing-in of air and the breathing-out of the gaseous products of combustion. This takes place in both animals and plants, and heat is the result. Hence when green vegetable matter composed of living cells is massed together these processes of respiration will continue for a time, and heat is the result. When such matter is massed together the heat evolved can not readily escape, and a considerable elevation of temperature is the result.

But the question may be asked, what causes the oxygen of the air to combine with the elements of organic matter whereby this combustion is affected. Under ordinary conditions oxygen has no affinity for organic carbon. Something must be present to stimulate this combination.

We have already found how water is made to combine with certain organic compounds through the agency of special enzymes, so in accordance with this it has recently been shown that a number of oxidation processes can be effected through the agency of another class of enzymes known as *oxydases*.

It has been already noted that yeast when in mass develops a rise of temperature, and it has generally been assumed that this is due to the respiratory activities of the cells, but it has recently been shown that there can be extracted from the yeast cells, independent of the cells themselves, a substance which has the power of oxidizing glycogen with a perceptible increase of temperature.

Thus it appears not to be the vital protoplasm of the cell but some substance which can be extracted therefrom which possesses the power of oxidizing organic matter with the production of heat. This active substance is an oxidizing enzyme.

It is now quite generally believed that oxidizing enzymes are quite generally distributed throughout vegetable tissues, and that they occur dissolved within the fluids of the cells.

In the presence of oxygen they cause a union of the latter with carbon, carbon dioxide being evolved. Thus it may be considered at least a working hypothesis that all processes of respiration are associated with the activities of oxidizing enzymes.

When green fodder is cut and placed in the silo, cells previously protected from the air are exposed, and the combined action of the air and the liberated oxidizing enzymes results in a rapid oxidation with loss of substance.

When fruits are cut open their exposed surfaces turn dark, due to the combined action of contained oxidizing enzymes and the atmosphere.

Besides the ordinary gaseous products of oxidation, it has been shown that oxidizing enzymes may produce certain by-products, notably the organic acids. Thus ensilage may become sour without a trace of bacterial fermentation.

Fresh olives when placed in heaps ferment. With this there is an increase of temperature, a liberation of carbon dioxide, and the formation of acetic and other fatty acids. Talomei shows this fermentation to be due to a special oxidizing enzyme which he called *olease*.

When green crops are plowed under their tissues continue to undergo an oxidation or respiratory process similar to that which

takes place in silage. The carbohydrates are mainly attacked with a certain loss of substance, the evolution of carbon dioxide and probably the production of organic acids. This process, however, does not continue long but is succeeded by the ordinary bacterial fermentation already stated.

5. The Decomposition of Proteid Matter.

Proteid matter is a valuable source of plant food because of its contained nitrogen. This nitrogen before it can be easily assimilated by the plant must be converted into the condition of nitrate. The stages leading up to the production of nitrates are:

1st. Putrefaction or the conversion of proteid matter into ammonia (ammonification).

2d. The oxydation of ammonia to nitrites, the first stage of nitrification, and,

3d. The oxidation of nitrites to nitrates, the final stage of nitrification.

These processes will be considered in turn.

(a) *Putrifaction and Ammonification.*

Liebig²³ and the older investigators considered putrefaction a chemical process, the final products of which were carbon dioxide, water and ammonia.

In 1837 v. Schwann made the important discovery that fermentation and putrefaction germs were invariably found in the atmosphere, and it was left to Pasteur and his co-laborers to demonstrate finally that putrefaction was due to the agency of micro-organisms.

Since the early discoveries of Pasteur it has been shown that a great variety of bacteria found in soil, water and organically polluted fluids are capable of effecting the decomposition of albuminous or proteid matter.

The first step in the change is the conversion of insoluble proteids into soluble peptones, a process similar to that which takes place in the stomach. The liquefaction, or as it is called, the peptonization of proteids is effected through the ability of the bacteria to secrete an enzyme of the nature of animal *trypsin*. All bacteria which liquefy gelatin have peptonizing properties to a greater or less degree, and hence the power of converting proteids into peptones. Liquefying bacteria are abundantly present in all soils, hence the vital agencies are there at work which cause a rapid peptonization of all proteid bodies.

The next step in the process is the conversion of peptones into amido-acids and basic amines.

The following is a list, after Rideal,²⁴ of the amido acids which have been found as products of the putrefaction of proteids:

Name.	Constitution.	Formula.	Products of Further Decomposition.
Glycocin,	Amido-acetic,	$C H_2 (N H_2) C O O H$,	Ammonia and acetic acid.
Leucin,	Amido-isocaproic, ..	$\left\{ \begin{array}{l} C_5 H_{10} N H_2, \dots\dots\dots \\ C O O H, \dots\dots\dots \end{array} \right\}$	Ammonia and isocaproic acid.
Tyrosin,	B-oxyphenol-amido propionic,	$\left\{ \begin{array}{l} C H_2 C_6 H_4 (O H), \dots\dots \\ C H_2 (N H_2) C O O H, \dots\dots \end{array} \right\}$	Indol, phenol and skatol.
Aspartic, ...	Amido-succinic, ...	$\left\{ \begin{array}{l} C H_2 C O O H, \dots\dots\dots \\ C H (N H_2) C O O H, \dots\dots \end{array} \right\}$	Ammonia and malic acid, then succinic.
Asparagin, .	Amido-succinamic, ..	$\left\{ \begin{array}{l} C H_2 C O (N H_2), \dots\dots \\ C H (N H_2) C O O H, \dots\dots \end{array} \right\}$	Ammonia and malic acid, then succinic.
Glutamic,	$\left\{ \begin{array}{l} C_3 H_5 (N H_2), \dots\dots\dots \\ C O O H, \dots\dots\dots \end{array} \right\}$	Ammonia and probably succinic acid.

The amido acids are next decomposed into ammonia and organic acids as shown in the last column of the above table. Tyrosin breaks into indol, phenol and skatol.

Of the basic products of putrefaction we have non-volatile bases known as ptomaines and leucomaines, produced in minute quantities, and certain volatile bases such as monomethylamine and trimethylamine. These latter basic products by further decomposition are converted into ammonia.

From the preceding it is seen that the final products of putrefactive fermentation are ammonia and organic acids. Naturally the organic acids will combine with the ammonia to form salts, but these salts will undergo a still further change in which the acid is converted into carbon dioxide, hydrogen and marsh gas. The two latter escape while the carbon dioxide combines with the ammonia to form carbonate of ammonia. This completes the process, the proteid matter resolving itself into two gases, hydrogen and marsh gas, with a solid residuum in the form of ammonium carbonate.

Theoretically this is true, but in reality there remains as a "by-product" of these reactions, as Rideal puts it, "a varying but small quantity of dark pulverent matter resembling the humus or peaty substances of soil."

In addition to this it is known that under certain conditions of exclusion of air, and of the development of the more strongly anaerobic bacteria, a certain amount of the nitrogen escapes in the free state before it is converted into ammonia. While this takes place in putrefying fluids such as sewage it probably does not occur to any appreciable degree in soils.

According to Sommaruga, aerobic bacteria growing in non-saccharine nutrient media always form an alkali from albuminous bodies. These alkaline bodies so far as known are either ammonia

or amides, which in part become converted into ammonia. Thus it may be said that ammonia production is almost a universal function of bacteria.

In the following table is shown the production of ammonia by several common species of soil bacteria grown in beef broth at room temperature:

Table III.

	Milligrams of NH_3 per 100 c. c. of Culture produced in			Percentage of the total nitrogen in the medium converted into ammonia in 30 days.
	Seven days.	Fourteen days.	Thirty days.	
<i>Bacterium mycoides</i> ,	9.18	20.06	45.50	21.9
<i>Bacillus subtilis</i> ,	6.46	18.35	46.20	21.4
<i>Bacillus pulvinatus</i> —				
Variety A,	1.02	10.20
Variety B,	5.78	18.30	8.4
<i>Bacillus</i> No. 6,	0.30	5.44	22.00	10.2
<i>Bacillus</i> No. 7,	2.40	19.72	38.10	17.6
<i>Microspira tenuis</i> ,
<i>Bacterium fermentationis</i> ,	0.30	8.50	27.90	12.9

In the above table it is seen that the highest quantity of ammonia was produced by *Bacterium mycoides* and *Bacillus subtilis*, the latter organism converting 24.4 per cent. of the total nitrogen of the medium into ammonia in thirty days. Both of these species are abundantly and constantly present in soils, and are important factors in the ammonification of organic matter.

It is also noted that there is a marked difference in the ability of the different species studied to produce ammonia, and in the rate of its development; one form *Microspira tenuis* producing none after a period of thirty days. Complete absence of ammonia production is however the exception.

(b.) Nitrification.

The subject of nitrification is one which has received a large share of attention from scientific men, and the literature thereon is very voluminous, extending over a period of twenty-five years.

In 1871-75, Sir J. H. Gilbert found that the drainage waters from

the experimental fields of Rothamsted contained more nitrates as the amount of ammonium salts applied to the soil increased.

In 1878 Messrs. Schlösing and Müntz²⁵ laid before the French Academy the results of an experiment tending to prove that nitrification was due to the action of an organized ferment. A glass tube one meter long was filled with ignited quartz sand and powdered limestone. Through this sewage was passed at intervals. During the first twenty days the sewage which passed the filter remained unaltered, after which nitric acid began to appear until the filtrate no longer contained any ammonium salt, but only nitrates. Thus it was shown that active nitrification was going on within the body of the filter, and it was suspected that micro-organisms so abundantly present in the same were the active agents of the change.

To demonstrate this point, chloroform vapor, a well-known germicide, was passed through the filter; as a result it was found that ten days after the introduction of the vapor all nitrates had disappeared, and the sewage passed through unchanged. In other words, the chloroform vapor had so paralyzed the micro-organisms present as to completely check the process of nitrification.

Messrs. Schlösing and Müntz were, however, unable to isolate any specific ferment capable of inducing nitrification, and nothing was accomplished toward this end until the year 1886. Celli-Zucco²⁶ and Heraeus²⁷ at this time succeeded in isolating from water rich in nitrates a number of forms of bacteria, which, however, only possessed very feeble nitrifying properties.

Frank²⁸ simultaneously with the latter attempted a similar isolation of the nitrifying organism, but without result, and concluded that nitrification was not due to the direct action of bacteria, but that it was a purely chemical process. This view was opposed by a number of writers, notably, Landolt, Platt and Baumann.²⁹

In 1888-89 Warrington³⁰ and also Frankland³¹ studied a large number of soil bacteria, but neither was able to find one which produced any thing approaching active nitrification. Frankland maintained, however, that the nitrifying organism was present, but had not been isolated.

In 1890 Frankland³² succeeded in cultivating a spherical (coccoid) organism about 0.8 micromillimeters in diameter, which possessed the power of converting ammonium salts into nitrous, but not into nitric acid. The separation was by means of the dilution method in media containing only inorganic salts. In this the form in question grew most successfully.

This fact, and other points in the investigation of Frankland, at once revealed the important principle that the organism of nitrification does not grow normally in media rich in organic matter, and that, therefore, the ordinary method of separation by means of

gelatin plates was inapplicable for the isolation of the specific nitrifying agent.

In the same year an identical principle was discovered and put into practice by Winogradsky³³ who succeeded in separating the nitrifying ferment by using a purely inorganic medium containing:

Water of Lake Zurich,1000 cc.
 Ammonium sulphate,1 gm.
 Potassium phosphate,1 gm.
 Basic carbonate of magnesia,an excess.

In this solution nitrification became very active, when previously inoculated with a small quantity of soil.

By a long series of fractional cultures one was finally obtained which contained but few bacteria except the nitrifying organism. From this somewhat impure culture, gelatin plates were made. On the principle that the foreign non-nitrifying organisms grow in gelatin while the nitrifying bacteria do not, an indirect method of isolation was utilized.

In the portions of the gelatin between the colonies of non-nitrifying bacteria the nitrifying organisms would be liable to be present in a pure state, but unable to produce colonies because of uncongenial soil. By removing bits of this apparently sterile gelatin a few nitrifying organisms, unmixed with others could be transferred to a favorable solution like the one already given. In this way Winogradsky was able to isolate the nitrifying organism.

Later, in 1891, Warrington,³⁴ in a solution containing mineral salts, obtained, after repeated generation, a culture which nitrified vigorously, and which, by containing no organism which would grow on gelatin, was regarded by him as containing only nitrifying bacteria. The germ thus obtained was an oval form seldom one micromillimeter thick and scarcely longer than broad.

At this time Winogradsky³⁵ made a decided improvement in the separation of the nitrifying organism from solutions containing it by the use of the Kühne gelatin silica medium.³⁶ The nutrient basis of this medium as used by Winogradsky was composed of: ammonium sulphate, 0.41 gram, magnesium sulphate, 0.05 gm., potassium phosphate, 0.10 gm., sodium carbonate, 0.6-0.9 gm., calcium chloride a trace, and water 100 cc.

The inoculation of the plates took place either by mixing the inoculating material with the above solution before the addition of gelatinous silica, or it was made as a streak or smear culture on the already hardened material. In this way the nitrifying organisms developed distinct colonies from which pure cultures were made.

The investigations of Winogradsky and simultaneously of Warrington showed:

1. That in the soil the nitrifying process was effected by two distinct but closely related organisms; the one converting ammonia into nitrous acid and nitrate, and the other changing the nitrites into nitrates.

2. That these two processes follow one another in such rapid succession that the production of nitrites is only a transitory phenomenon, so that if both the nitrite and nitrate organism be added to sterilized soil the process is completed in the natural way, only the merest traces of nitrous acid appearing.

The nitrate organism of Winogradsky is an oval form about 0.5 micromillimetres in length. The nitrite germ varies from oval to spherical and is about twice the size of the former. See Figs. 5 and 6.

If to a mineral solution containing ammonium salts, a pure culture of nitrite ferment be added, only nitrites will be formed, and these will remain unchanged in the absence of the second nitric ferment. If, however, the two organisms be added simultaneously, nitrates will be rapidly formed.

In 1892, Winogradsky³⁷ studied the nitrifying organisms of soil, from a number of different localities. Those from several parts of Europe, from Africa, and from Japan, which he considers to be the same organism, he names *Nitromonas europea*; a second form from Java soil, differing from the first he names *N. javanensis*. Both of these comprise the nitrite ferments of Winogradsky; the second nitrate ferment was isolated by Winogradsky from Quito soil and differs from the first not only as to size, as above mentioned, but also by entirely lacking the motility common to the latter.

The notable researches of Winogradsky have been followed by others which have interest from a controversial standpoint.

In 1895 Burri and Stutzer³⁸ isolated from soil a nitrate organism with properties akin to the Quito bacillus of Winogradsky. It was a motile organism, 0.75-1.5x0.5 micromillimeters, growing on silica plates in definite colonies, but also possessing the power to grow on gelatin and to liquefy the medium; said organism according to the writer being able to convert nitrites into nitrates, but losing such power when grown on organic media.

The above results of Burri and Stutzer, so contrary to those of Winogradsky, brought forth a vigorous rejoinder from the latter. In this Winogradsky stated that he tested the same earth used by Burri and Stutzer and isolated therefrom his own *Nitromonas*, and that the latter when tested in bouillon, meat pepton, gelatin and agar failed to grow. He therefore regards the German work as erroneous.

In 1897 Stutzer and Hartleb⁴⁰ appeared with a still more startling series of discoveries in which they not only maintained the ability of the nitrifying organisms to grow in organic media, but also showed

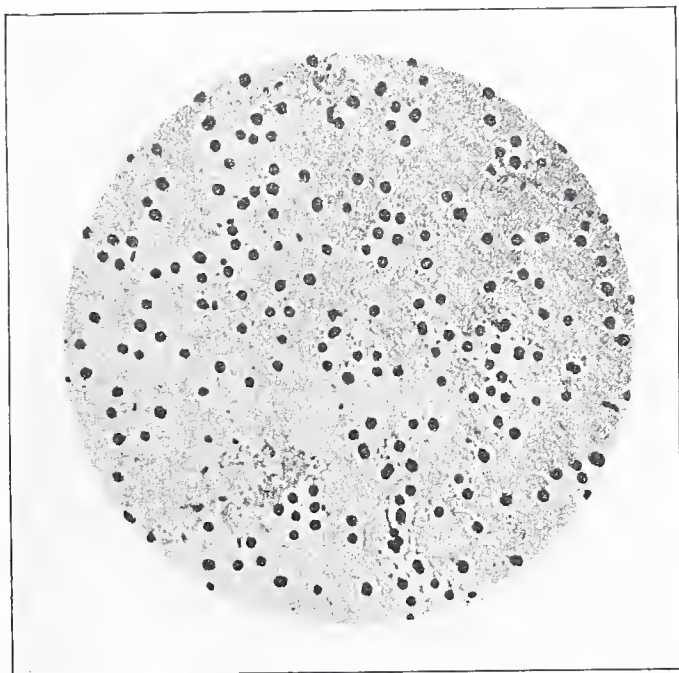


FIG. 5.—The Nitrite Organism of Winogradsky, isolated from Quito earth. Converts ammonium salts into nitrites, the first stage of nitrification. (After Winogradsky: *Ann de l' Institut Pasteur*, 1891, Plate XVIII.)

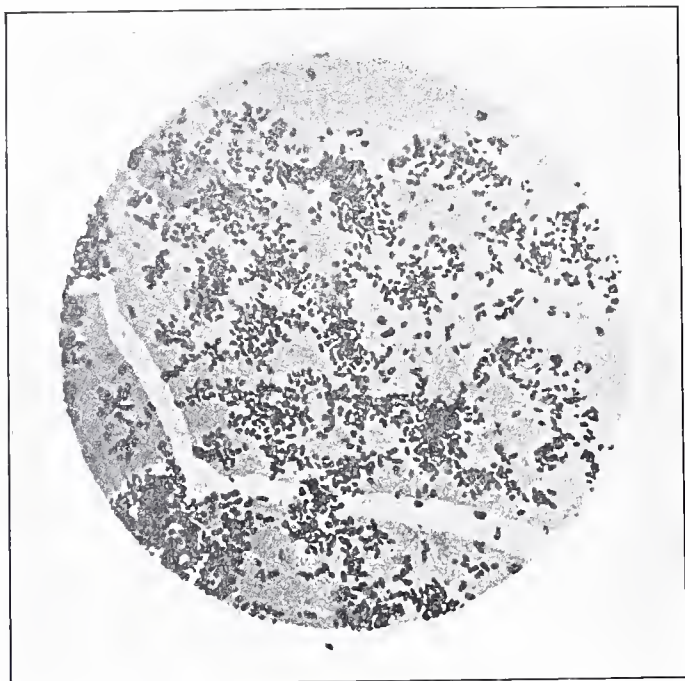


FIG 6.—The Nitrate Organism of Winogradsky, isolated from Quito earth. Converts nitrites into nitrates—the second stage of nitrification. (After Winogradsky: *Ann. de l' Institut Pasteur*, 1891, Plate XVIII.)

that the latter possessed a polymorphic habit never before imagined in this or any other like group in the whole domain of mycology. The ability of simple coccoid or rod-shaped forms to develop into filaments or even into branched forms, with the further production of true gonidia and other even more highly organized fructification bodies, is a species of morphological transcendentalism likely to cause skepticism.

I will not enter into the details of Stutzer and Hartleb's investigations, as I think we should await further researches before giving them credence. It is easy to believe that the latter's culture, which they claimed to be those of the organism of nitrification, were in reality impure, and that in the variable forms described by them they were in reality dealing with several distinct organisms instead of one.

That this seems true is indicated by the investigations of Gärtner⁴¹ and Fräenkel⁴² during the present year.

Gärtner discusses the work of Burri, Stutzer and Hartleb on the polymorphism of the nitrifying organism, and from presumably pure cultures of the latter's nitrifying ferment was able to isolate thirteen different micro-organisms, including a fungus form (Schimmelpilz); thus proving their impure character. Furthermore, Gärtner showed that these several organisms, when once separated in their pure state, retained their fixed character, with no tendency to polymorphism, and indicated none of those transition stages from bacteria to fungi noted by Stutzer. Again none of these isolated organisms possessed the power to convert ammonia into nitrites. C. Fraenkel simultaneously isolated from Burri and Stutzer's cultures 11 different organisms, including 7 bacilli, 2 streptothrices and 2 fungi (a Fadenpilz and a Schimmelpilz). These showed no polymorphism, but all retained constant characters.

From what has been written there is reason to believe:

1. That nitrification in the soil is caused by a distinct or rather by two distinct organisms possessing certain definite characters.

2. That these organisms will not grow in the presence of any considerable amount of organic matter, and that all reported attempts to cultivate them on ordinary organic media are without authentication.

3. That the above nitrifying organisms are found abundantly in all cultivated soils, and that in ordinary soil water containing, besides mineral salts, a due proportion of ammonium carbonate, sulphate, etc., they find a favorable medium for their development.

4. That the result of such development is: (a) The conversion of ammonia into nitrous acids through the agency of the nitrous organism; and (b), the immediate conversion of the previous nitrous into nitric acid by means of the equally abundant nitric ferment.

Understanding, therefore, that these two stages of nitrification are

due to definite organisms in the soil, the laws which control nitrification are simply the laws which control the life and development of said organisms.

If a soil be wet with a one per mille solution of bichloride of mercury, no nitrification can take place; firstly, because the mercury compound has destroyed the life of all nitrifying as well as other organisms, and secondly, because the presence of such a salt in the soil will prohibit the development of all nitrifying ferments which come after.

Again, if a soil be heated above a certain temperature, all nitrifying ferments will be destroyed and nitrification will cease.

Chloroform, formaldehyde gas, sulphurous acid gas, etc., passed through a layer of soil will, on the same principle, prevent further nitrification.

These, however, are all agencies implying the total destruction of the nitrifying ferments. In addition to these there are certain conditions which, while they do not annihilate the life of the nitrifying organism, are yet either favorable or unfavorable to their normal development. These conditions the agriculturist must understand, in order to control the nitrifying process in the soil.

(c.) Conditions Affecting Nitrification.

Quantity of Organic Matter or Humus in the Soil.

It has already been stated that the nitrifying organism will not grow in a medium containing any considerable amount of soluble organic matter.

When sewage is applied to land nitrification cannot, therefore, begin until after the ordinary putrefying and ammonifying bacteria have decomposed much of the organic matter and rendered the soil water a fit pabulum for the development of nitrifying ferments.

In a 12 per cent. solution of urine, according to Warrington,⁴³ nitrification did not begin until about ninety days, and then only after 447 milligrams of ammonia per litre had been produced by the decomposition of the organic matter present.

For this reason sewage when applied to land should be used cautiously and not too frequently, otherwise the soil will become so rich in soluble organic matter as to hinder nitrification and thus render the large accessions of nitrogenous food useless.

Thus a soil too rich in humus is an unfavorable nitrifying ground up to the limit when nitrification ceases. Just what this limit is, is as yet unknown. Wiley⁴⁴ mentions a soil from Florida containing over 80 per cent. of organic matter, and less than 10 per cent. of sand and other mineral matter, which was found to be entirely free from nitrifi-

fyng ferments. In ordinary arable land, however, the quantity of organic matter, or humus, never reaches a height sufficient to interfere with the process of nitrification, and hence only in rare exceptions does this factor enter into the question of nitrification.

Aeration and Cultivation.

The nitrifying ferments are what are technically called aerobic, that is, they grow only in the presence of atmospheric air.

Schlösing⁴⁵ has determined the rate of nitrification in a moist soil in an atmosphere containing various proportions of oxygen as follows:

1. When oxygen was entirely absent there was a reduction of nitrates and an evolution of free nitrogen.
2. With 1.5 per cent. of oxygen nitrification was marked.
3. With 6 per cent. of oxygen the quantity of nitrate produced was more than double the above (2).
4. With 16-24 per cent. of oxygen the quantity of nitrate produced was more than four times that in (2).

The effect of stirring and pulverizing the soil upon the growth of crops has long been known, and long before the philosophy of the operation itself was in any wise understood.

Among other reasons why cultivation is beneficial is the fact that it aids nitrification by bringing the oxygen of the air into more immediate contact with the nitrifying ferments. The effect of stirring and pulverizing the soil is well shown in an experiment by Deheraine.⁴⁶

Six pots filled with soil, which had remained undisturbed for two years, were selected. Three of the pots had their soil emptied on a clean floor in separate piles, and each lot was kept stirred and pulverized from time to time for a period of six weeks. In three of the pots the soil remained untouched. At the end of the period the nitrates in the several soils were determined, when it was found that the average percentage of nitrates in the three stirred and pulverized soils was 23.7 times that found in the soils which had remained undisturbed in the pots. While it is not presumed that ordinary farm cultivation will produce as marked results as the preceding experiments indicate, it is, nevertheless, clear that the effect of cultivation is to markedly increase nitrification, and in a ratio proportionate to its thoroughness and frequency.

In this principle we have the explanation of why the late cultivation of orchards is inadvisable. Nitrification is more active in autumn than at any other time. This activity would be increased enormously by cultivation, resulting in the production of large

stores of nitrates, the bulk of which would be washed out of the soil by the winter and early spring precipitation.

The Presence of Moisture.

Bacteria of all kinds, including the nitrifying organisms, grow only in the presence of moisture. Soil waters, containing dissolved mineral salts and ammonium compounds, are, therefore, the natural media in which the nitrifying ferments develop.

In a dry soil nitrification cannot take place; hence in periods of drouth, where the superficial layers of the soil for a depth of several inches become dry, nitrification is suspended. On the other hand, excess of water prevents nitrification by excluding air, hence water-logged soils must first be drained before they become proper nitrifying beds. In a wet soil not only is nitrification inactive or entirely suspended, but the opposite process of denitrification takes place with the loss of nitrogen in the free gaseous condition.

A soil may be in good condition in its superficial layers, but have an impervious clayey subsoil, which retains the downward drainage; or a still deeper layer of clay may raise the level of ground water so near the surface as to render the subsoil wet. In either case the deeper zones of the soil become too damp for active nitrification, so that this process instead of being carried on from the surface to a depth of about 27-36 inches is confined to a more superficial zone; the elaboration of much valuable plant food is thereby prevented.

The functions of underdrains is thus not only to withdraw the excess of subsoil water, but also by the downward movement of the same to draw air into the soil and thus supply oxygen to the nitrifying ferments.

The Reaction of the Soil.

Nitrification can only take place in a feebly alkaline medium, but the presence of anything beyond a small quantity of an alkaline salt is a hinderance to the process, while a large amount will check it entirely. Thus Warrington⁴⁷ showed that the presence of 0.032 per cent. of bicarbonate of soda distinctly retarded nitrification, and that with the presence of 0.096 per cent. nitrification was only barely possible. The same author also showed that the presence of 0.0447 per cent. of ammonia in urine rendered it unnitrifiable. Dumont⁴⁸ and Crochetelle showed that carbonate of potash added to soil at the rate of from 1 to 2.5 grams per 1000 grams of soil markedly increased nitrification, but that large applications of the salt progressively diminished the rate of nitrification, and that the addition of 8 grams per 1000 grams of soil completely checked it.

A heavy dose of lime by unduly increasing the alkalinity of the

soil may at first check or suspend nitrification until the said lime has been converted into carbonate. This, however, takes place rapidly, diminishing in turn its strong alkaline properties and permitting nitrification to commence more actively than before.

Equally unfavorable to nitrification is an acid condition of the soil. The extreme susceptibility of the nitrifying organism to acid is shown by the researches of Wiley and Ewell.⁴⁹ In the experiments of the latter, solutions containing calcium chloride and water were seeded with soil, containing of course nitrifying bacteria. In this medium nitrification continued until the medium reached an acidity equivalent to 4 c. c. of normal acid per 100.

It is a well known fact that nitrification has practically ceased in forest and woodland soils. The same is true, but to a lesser degree, in the soil of old pastures. That such cessation of nitrification is not due to the absence of nitrifying bacteria is shown by the fact that such soils begin to nitrify rapidly upon the addition of some base which overcomes the acidity.

In the experiment of Dumont and Crochetelle, already referred to, a soil which had been in grass from time immemorial and contained 6.84 per cent. of humus was treated with variable amounts of carbonate of potash. It was stirred and watered several times during the experiment. After one month the nitrates were extracted with the following results: Nitric nitrogen, per 1000 grams of soil, without addition of carbonate of potash, 70 mg; with 1 gram of carbonate of potash per 1000 grams of soil, 160 mg; with 2 grams of carbonate of potash, 230 mg; with 3 grams, 250 mg; with 4 grams, 130 mg; with 5 grams, 73 mg.

In an experiment with marsh land containing 5.76 per cent. of humus similar results were obtained, showing that while in the original soil nitrification was practically *nil*, the addition of carbonate of potash resulted in active nitrification.

Too great a degree of alkalinity, as has been already seen retards or entirely checks nitrification.

In a too concentrated solution of urine, the nitrifying process is hindered after a certain time by the accumulation of ammonium carbonate which is being produced by the ammonifying bacteria at a more rapid rate than the oxidation of the ammonia to nitric acid. This is the condition of most stable manures in bulk or before they become intimately mixed through the soil.

The addition of lime to stable manure in a state of ammoniacal fermentation would only still further hinder their nitrification by increasing alkalinity, and would also drive off much valuable ammonia. Lime added to fresh manure before ammonium carbonate has been produced will not cause the above loss of free ammonia,

but it will hasten the ammoniacal fermentation so rapidly that nitrification will soon cease.

Both ammonification and nitrification can be made active in a manure heap, when not too closely compacted, or in a soil freshly and heavily dressed with manure, by the use of plaster. Plaster acts by decomposing the excess of ammonium carbonate as fast as formed, producing in turn non-volatile ammonium sulphate, and calcium carbonate (chalk); the latter compound serving as a mild base whose presence is favorable to nitrification.

The loss of ammonia from manure piles is not considerable as long as the latter are kept moist or well compacted. When manure becomes dry, or when the latter is forked over or otherwise kept too loose, a considerable loss of ammonia will result. So far as the loss of ammonia alone is concerned, the application of plaster may not be necessary, but if the latter be spread over the pile with each considerable addition of manure from the stable, its presence will materially aid in the ammonification and nitrification of the same and make it a more active stimulant when applied to the land.

Temperature of the Soil.

Experience at Rothamsted in England shows that nitrification takes place quite freely in the soil during an ordinary English winter. Warrington⁵⁰ in one series of laboratory experiments showed a considerable rate of nitrification in solutions kept at the temperature of 37 and 39 degrees F.

According to Schlösing and Müntz, nitrification becomes active at 54 degrees F. and rapidly increases up to 99 degrees F., at which point it is nearly ten times as rapid as at 54 degrees. Above 99 degrees the rate of nitrification rapidly diminishes; at 122 degrees F., very little nitrate is produced, and at 131 degrees F., it ceases entirely.

The Physical Condition of the Soil.

Schlösing has shown that nitrification and microbial combustion in general are less active in fine-grained compact soils than in lighter coarse-grained soils.

A certain proportion of clay and sand appears to be most favorable for a maximum rate of oxidation. In the experiment of Schlösing equal amounts of ammonium sulphate were applied to equal quantities of artificial soil in pots, and the nitrates determined at the end of certain periods; from this the percentage of the ammonium salt which was converted into nitric acid was determined as given in the following table:

Table IV.

	Composition of Soil.		Percentage of the ammonium salt nitrified at 20°-27° C. after 72 days.
	Sand.	Clay.	
Soil No. 1,	100	83
Soil No. 2,	90	10	94
Soil No. 3,	80	20	89
Soil No. 4,	75	25	56
Soil No. 5,	70	30	10
Soil No. 6,	100	*63
Soil No. 7,	90	10	*66
Soil No. 8,	85	15	*94
Soil No. 9,	80	20	*100
Soil No. 10,	75	25	*21
Soil No. 11,	70	30	*2.7

*Percentage of ammonium salt nitrified after 106 days, temperature of laboratory.

From this table it is seen that the maximum rate of nitrification took place in the soil with a proportion of clay varying from 10-20 per cent. with from 80-90 per cent. of sand respectively, the rate diminishing as it is either lighter or heavier than the above mean. Nitrification, other things being equal, is, therefore, most active in soils which are neither too light nor too heavy.

The soil best meeting these conditions are the sandy loams which occupy an intermediate position between the heavy grass lands of the inner margin of the costal plain and the light truck lands of the sea-board. One way in which soil texture affects rate of nitrification is by its effect on available water.

The finer particles of heavy soils have such a strong attraction for water that a large part of the soil moisture is rendered unavailable for the use of the nitric ferment.

(d.) *The Rate of Nitrification in the Soil.*

When samples of soil are taken and kept loosened and stirred, and under favorable conditions of temperature and moisture, the rates of nitrification therein are exceedingly high, as shown by the following Table V⁵⁰ from experiments by Lawes and Gilbert:

Table V.

Source of Soil.	Parts of nitric nitrogen produced per day per million parts of air-dry soil.	Period of experiment in days.
Rothamsted soil, first 9 inches,	0.588	119
Do. with ammonium chloride added,	0.944	119
Manitoba soils, Lawes and Gilbert,	0.700	335
Soil, Dehérain,	0.76-1.09	90

The large quantities of nitrates produced in the rich Manitoba soils is due to the relatively large amounts of humus which they contain; the figures for the Rothamsted soil in the same table will, therefore, represent a more normal and average condition.

More detailed figures regarding rates of nitrification are given in Table VI⁵¹ for one of the poorest of the Manitoba soils.

Table VI.

Depths—Inches.	Nitrogen as Nitric Acid Produced per Day During Different Periods of Exposure, in Pounds, Per Acre.						Total nitrogen produced in 285 days.	Total nitrogen per acre in original soil.	Percentage of nitrogen converted into nitric acid in 285 days.
	Periods of Exposure.								
	1st.	2d.	3d.	4th.	5th & 6th.	7th.			
1-12,	0.92	0.88	1.08	1.00	0.36	0.69	227.	5,236	4.78
13-24,	0.17	0.12	0.12	0.52	0.16	0.27	57.2	3,488	1.64
25-36,	0.58	0.49	0.03	0.18	0.03	0.03	43.8	2,592	2.07
37-48,	0.08	0.04	0.01	0.01	0.02	0.02	7.6	870	1.22
							335.6	12,186	

The preceding table shows:

1. That the greater proportion of the nitrates, nearly 65 per cent., are produced in samples from the first 12 inches of soil; both because of the greater quantity of humus in the surface layer and perhaps also because of the greater vigor of the nitrifying ferments in this same zone.

2. That in zones between 12 and 36 inches below the surface large quantities of nitrates can be found, 30 per cent. of the whole, pro-

vided samples from this depth are brought under conditions favorable for nitrification.

3. That at depths greater than 36 inches the proportion of nitrates which can be found is small.

The large quantities of nitrates produced in 285 days, calculated as 335 pounds per acre, in terms of nitric nitrogen, show that our soils when brought under the most favorable conditions for nitrification can be made to produce nitrates at a rate far in excess of the needs of the most exacting crops.

Again, the small proportion of nitrogen converted into nitric acid in a single year, as compared with the total nitrogen of the soil, as shown in the last column of Table VI, demonstrates, furthermore, that virgin soil will at least become abundant sources of nitrogen to growing crops for scores of years.

The question of nitrogen supply in even ordinary soils becomes, therefore, a question of controlling nitrification and of conserving the supplies of nitrates produced by nature. It is not to be supposed, however, that anything like the supplies of nitrates obtained in the preceding experiments can be realized by ordinary tillage to the depths indicated. The production of an amount of nitric nitrogen in any wise approaching 335 pounds to the acre is, therefore, not to be expected. Were it so nature would be most wasteful in her operations, a charge which can never be truthfully brought against her.

A crop of wheat at the rate of 15 bushels of grain per acre will require something like 24 pounds of nitrogen; therefore to produce normal crops the economy of nature would demand that amounts of nitric nitrogen comparable to the actual needs of crops should be produced. This would be true to an approximate degree, provided the roots had complete possession of the land, down to the lower limits of nitrification, and also provided such crops had possession of the land during every month of the year.

In the case of wheat for instance, neither condition holds and consequently the crop being unable at all times to utilize nitrates as fast as they are produced, there must needs be considerable loss.

The fact that the drainage waters from unmanured wheat fields show an amount of nitric nitrogen approximately equal to that used by the crop is an indication that there is produced in the soil each year a stock of nitrates more than double in quantity that which the crop itself can utilize.

The relation between the quantity of nitrogen removed by a crop and that present in the drainage water is brought out in the following Table VII by Lawes and Gilbert. The figures show (1) the number of pounds of nitrogen per acre in crop and in drainage water;

(2) the number of parts of nitrogen in crop and in drainage compared with 100 parts in the manure. These last figures are given in black-faced type. The figures in the last column give the amounts of nitrogen unaccounted for, the plus sign indicating a gain of nitrogen and the minus sign a loss of nitrogen over that contained in the manure.

Table VII.

Plot.	Character of Manuring.	Nitrogen in Manures.	Nitrogen in Crop.	Nitrogen in Drainage.	Nitrogen Unaccounted for.
3 and 4,	Manured continuously,	0 lbs. per acre,	12 lbs. per acre,	15 lbs. per acre,	+27 lbs. per acre.
16,	Unmanured since 1865-1882,	0 per cent.,	45.5 per cent.,	55.5 per cent.,	+100 per cent.
5,	Mixed mineral manures,	0 lbs. per acre,	46.6 per cent.,	53.3 per cent.,	+30 lbs. per acre.
6,	Mixed +200 lbs. ammonium salts,	0 per cent.,	48.5 per cent.,	51.5 per cent.,	+100 per cent.
7,	Mixed +400 lbs. ammonium salts,	44 lbs. per acre,	61.3 per cent.,	50.0 per cent.,	+5 lbs. per acre.
8,	Mixed +600 lbs. ammonium salts,	88 lbs. per acre,	45.4 per cent.,	31.8 per cent.,	+11.3 per cent.
9,	Mixed +550 lbs. nitrate of soda,	132 lbs. per acre,	49 lbs. per acre,	43 lbs. per acre,	-20 lbs. per acre.
10,	400 lbs. ammonium salts alone,	86 lbs. per acre,	37.1 per cent.,	32.5 per cent.,	-22.8 per cent.
11,	400 lbs. ammonium salts + super-phosphate,	88 lbs. per acre,	37.2 per cent.,	67.4 per cent.,	-30.4 per cent.
		100 per cent.,	14 lbs. per acre,	50 lbs. per acre,	+46 per cent.
		100 per cent.,	15.9 per cent.,	56.8 per cent.,	-24 lbs. per acre.
		100 per cent.,	32.9 per cent.,	39 lbs. per acre,	+27.3 per cent.
				44.3 per cent.,	-30 lbs. per acre.
					-22.8 per cent.

The preceding table shows from 50 parts of nitrogen in the drainage as compared with 61.3 parts in the crop up to 67 parts in the drainage as compared with 37.2 parts in the crop.

The table also shows that in the case of wheat the greater proportion of the nitrates in the soil are either beyond the reach of the roots, or what is more probable, that they are washed out of the soil at that season, fall or winter, when the wheat has but limited capacity of absorbing them. For this reason wheat is shown to be one of the most exhaustive of all farm crops as regards nitrogen supply. That no such proportionate loss of nitrates occurs with other crops, like grass and clover, which cover the ground especially during the fall and winter months is shown by numerous figures, some of which will be considered under the head of loss of nitrates.

(c.) *Losses of Nitrates.*

Relation to Rainfall and Percolation.

Nitrates are easily leached out of the soil and carried into the drainage. In a highly porous, sandy soil free of vegetation, the greater proportion of the annual rainfall, 80 per cent. more or less, will pass downward through the soil and appear as drainage.

In land covered with a sod a much less percentage, about one-third of the rainfall, may pass off as drainage, the remainder being evaporated or thrown off by the plants in transpiration.

In a plot of fallow ground at Rothamsted⁵² for a period of 28 years the annual average outflow of drainage was 11.76 inches and the average rainfall for the same period 28.3 inches, making the average percentage of drainage 41.5 per cent. of that of the rainfall.

The action of vegetation in preventing an excessive outflow of drainage is most evident during the months of maximum growth May to September, as the following figures by Greaves and Evans⁵² indicate:

	Rainfall—Inches.	Drainage.	
		Inches; Sand.	Inches; Sod.
January,	2.87	2.73	2.03
February,	1.59	1.52	1.08
March,	1.93	1.60	0.88
April,	1.43	1.12	0.27
May,	2.05	1.65	0.10
June,	2.20	1.57	0.15
July,	1.77	1.21	0.01
August,	2.33	1.78	0.11
September,	2.35	1.74	0.07
October,	2.73	2.40	0.51
November,	2.02	1.96	0.83
December,	2.42	2.17	1.51
Total,	25.7	21.5	7.6

It is evident that the larger the rainfall the greater will be the amount of drainage, and the greater *pari passu* the loss of nitrates. Another factor also governs the amount of nitrates in the drainage, and that is the quantity of nitrogen in the soil, particularly in its soluble form.

The application of considerable quantities of ammonium salts or of nitrates as fertilizers, or of nitrogenous manures as stable manure or rape cake, results in a large increase of nitrates in the drainage. These facts are brought out in the following table from Lawes and Gilbert:

Table VIII.

Nitrogen as Nitrates in Drainage Waters, Broadwalk Field at Different Seasons. Average of three Years in parts Per Million of Drainage Water.

Plot.	Spring sowing to end of May.	June to harvest.	Harvest to autumn sowing.	Autumn sowing to spring sowing.	Whole year.	Nitrogen per acre per inch of drainage.	Character of the Manuring of the Land.
5,.....	2.9	0.2	4.8	5.5	4.2	0.95	Mixed mineral manures.
6,.....	14.7	0.7	6.0	5.4	5.4	1.22	*200 lbs. ammonium salts and minerals.
7,.....	27.1	1.4	7.3	5.4	6.8	1.54	*400 lbs. ammonium salts and minerals.
8,.....	28.2	4.0	13.5	7.5	9.3	2.10	*600 lbs. ammonium salts and minerals.
15,.....	6.7	2.9	7.5	28.1	19.3	4.37	†400 lbs. ammonium salts and minerals.
17,.....	29.7	1.8	6.6	5.5	7.1	1.61	400 lbs. ammonium salts alone.
18,.....	1.5	0.3	5.6	5.5	4.3	0.97	Mixed mineral manure.
2,.....	3.6	1.4	6.0	9.5	7.5	1.70	Barn yard manure.
19,.....	3.7	0.5	7.0	14.0	10.4	2.35	Rape cake.

*Applied in the spring.

†Applied in the fall.

The use of large quantities of ammonium salts, or of nitrate of soda at the time of spring seeding results, as the last table shows, in an immediate increase of nitrates in the drainage waters.

There is every reason to believe that ammonium salts, when applied to the soil, are very rapidly converted into nitrates, and in this form washed out of the soil. The same loss would follow similar applications at the time of autumn sowing as shown by the results from Plot 15, given in the last table.

Hence whenever it becomes necessary to use ammonium salts or nitrates as a crop stimulant, they should be applied in small quantities while the crop is growing. The custom of introducing nitrate of soda with the seed is accordingly a most wasteful operation.

The Amounts of Nitrates Lost in the Drainage.

It is calculated that the River Rhine discharges daily into the ocean 220 tons of nitrates, calculated as nitrate of soda; the Seine 270 tons, and the Nile 1,100⁵³ tons.

Since the great bulk of this comes from nitrates produced in the soil it is easy to form some idea of the tremendous losses of this the most valuable of all plant nutrients.

From what has already been said it is seen:

1. That given equal rainfall, the amount of nitrates lost in the drainage is greater in sandy than in heavy soils; and in direct ratio to the porosity of the latter.

2. That the loss by drainage, and hence the corresponding loss of

nitrates, is diminished by vegetation; hence in fallow land the loss of nitrates is greater than in land covered with plant growth.

Thus from an unmanured field at Rothamsted, kept fallow and free of weeds, the loss of nitric nitrogen per acre per annum was for three successive years 38.9, 48.3 and 27.4 pounds respectively, while from an experimental wheat field at the same place the similar loss, as an average of 19 years, was in one case 9.1 and in the other 11.9 pounds per acre⁵² per annum.

The Effect of Mineral Fertilizers on the Loss of Nitrates and on Nitrification.

I have already mentioned the effect of lime and plaster on nitrification; still another point needs mention.

The nitrifying organism cannot multiply except in the presence, among other elements, of phosphoric acid and potash. Nitrification is, accordingly, aided by applications of mineral fertilizers.

The effect of potash salts alone, or of potash salts mixed with carbonate of lime, in increasing nitrification and ammonification in soils rich in humus, has been shown by Dumont.⁵⁴ In soils differently treated the amount of nitric nitrogen produced in 1000 grams of soil in 40 days was in milligrams as follows:

Check,	2.8
Potassium carbonate, 0.1 per cent.,	57.8
Unleached ashes, 0.5 per cent.,	19.0
Muriate of potash, 0.1 per cent., and carbonate of lime, 2 per cent.,	38.0
Muriate of potash, 0.1 per cent., and Thomas slag, 5 per cent.,	41.5
Carbonate of lime, 2 per cent.,	5.3

From the preceding it is seen that a marked increase of nitrates resulted when some form of potash was used. In the mixture of muriate of potash and carbonate of lime, a double reaction between the two took place, producing carbonate of potash and chloride of calcium. The author claims that the action of the potash salts is to combine with the humates of the soil and form a compound which is very readily nitrifiable.

Again, the ability of a crop to utilize the nitrates of the soil is considerably diminished when there is a deficiency of available mineral constituents, especially of potash and phosphoric acid.

The effect of mineral salts upon nitrification and the loss of nitrates is well shown in the following table:⁵³

Table IX.

Nitrogen as Nitrates in Soil and Subsoil and in the Drainage Water of Various Plots in the Broadwalk Wheat Field, in Pounds Per Acre.

Plot.	Nitric Nitrogen in the Soil— Pounds Per Acre.				Nitric nitrogen removed by drainage Aug. 30- Jan. 31—lbs. per acre.	Percentage of total nitro- gen of soil removed by drainage.	Character of Manuring.
	1st—9 inches.	2d—9 inches.	3d—9 inches.	Total 27 inches.			
2,.....	9.7	5.3	2.8	17.8	11.6	65.1	Unmanured.
5 a,.....	12.6	7.1	4.6	24.3	13.2	54.3	Mixed mineral manure.
10 a,.....	14.2	11.9	7.3	33.4	31.1	93.1	400 lbs. ammonium salts.
7 a,.....	22.3	11.3	5.7	39.8	24.1	60.5	do. + mixed minerals.
11 a,.....	17.9	9.3	3.6	30.8	27.5	89.2	do. + super-phosphate.
8 a,.....	21.1	13.9	7.8	42.8	30.1	70.3	600 lbs. ammonium salts and mixed minerals.

From the above we note that the total nitric nitrogen in 27 inches of unmanured soil was 17.8 pounds per acre; while in the same soil, treated with mixed mineral fertilizers, the amount was 24.3 pounds per acre.

The percentage of nitric nitrogen which passed out in the drainage was also diminished as a result of the application of mineral fertilizers.

The effect of mixed minerals, when applied with ammonium salts in diminishing the loss of nitrates in the drainage, is well shown in the results from Plot 10a, 7a, and 8a, in the preceding table.

The Effect of Season of Year Upon Loss of Nitrates.

The preceding table shows that in unmanured land, and in land to which only mixed fertilizers, free from nitrogen, had been applied the percentage of nitric nitrogen removed by the drainage from September 1 to February 1, five months, was from 54 to 65 per cent. of the total quantity present in the first 27 inches of soil at the beginning of the experiment. In Table VIII similar results are observed.

On Plot 5, fertilized by non-nitrogenous manures, the average number of parts of nitrates per million parts of drainage for the whole year was 4.2 while the corresponding figures for the periods from wheat harvest to autumn sowing, and from autumn sowing to spring sowing, were respectively 4.8 and 5.5. The corresponding figures for the periods from spring sowing to the end of May, and from June to harvest, were 2.9 and 0.2.

The losses of nitrate in a wheat field, or on fallow ground are therefore greater during the fall and winter months; and least during the summer months.

The increased loss of nitrates in a wheat field during the fall and winter months is due to a combination of causes:

1. Diminished evaporation and increased drainage.
2. The accumulation of nitrates in the soil during the summer months beyond the needs of the plants, which are washed out of the same during the fall and winter, and
3. The inability of wheat at this season to utilize the soil water and prevent excessive percolation.

These considerations teach a most important principle, i. e., that ground should be kept in some crop as much of the time as possible especially during the fall and winter. The growth of wheat as one crop in a system of rotation is of course necessary, notwithstanding the inevitable losses of nitrates which follow its seeding.

There are, however, certain violations of the above rule which need correction.

Corn land should never be left fallow through the winter. The same is equally true of tomato and trucking land. Either these crops should be followed by wheat or some winter cover crop put in to conserve nitrates. In a loose sandy soil in which it is more difficult to accumulate available nitrogen it would be inadvisable to follow a cultivated crop like tomatoes, or potatoes by wheat, but rather to use crimson clover or rye to hold the nitrogen.

(f.) Increasing the Supply of Available Nitrogen in the Soil.

Soils may become too rich in humus and available nitrogen. The use of crimson clover has in some cases in Delaware been carried so far as to work actual injury to the land, especially if the latter has a tendency to become heavy and retentive of nitrates. The majority of farm lands, however, are not open to the charge of being too rich; on the contrary, the improvement of land and the growth of larger crops is the great desideratum.

I have already pointed out that most soils contain large supplies of organic nitrogen, which, by the aid of nitrification, can be made available to crops. The question of utilizing these stores of organic nitrogen already in the soil becomes mainly one of underdraining, deep plowing and more frequent cultivation.

Every cultivation of a corn or potato crop is equivalent to a dressing of nitrate of soda in its cheapest possible form. Hence if we could cultivate twice to each once by our present system we would

considerably increase our supplies of available nitrogen, and in turn reap the rewards of such an increase in larger crops.

Such a system of intensive cultivation carried on year after year would, however, result in the burning out of the land, and in greatly reducing the fertility. It is, therefore, necessary to make good these losses of organic nitrogen by the growth of such crops, or by the use of animal manures, as shall add to the stock of humus already in the soil. The effect of stable manure and clover in increasing the quantity of nitrates in the soil is brought out in the following table:³¹

Table X.

Nitrogen as Nitric Acid in Pounds per Acre in Soils of Geescroft and Hoosfield Experimental Plots, Rothamsted.

Depth—Inches.	I.	II.	III.	IV.
	Geescroft field, land in beans 30 years continuously, then 4-5 years fallow without manure.	Geescroft field as in I. Farmyard manure.	Hoosfield, wheat and fallow alternately for 35 years.	Hoosfield, 17 years in clover, 12 years fallow and six crops grain in 35 years. No nitrogen manure.
1-9,	4.28	13.57	19.85	30.90
10-19,	5.52	8.76	8.05	27.73
19-27,	4.81	7.70	2.47	8.44
28-36,	2.69	8.51	2.70	7.64
37-45,	2.68	4.36	1.62	9.07
45-54,	1.90	1.85	3.57	8.77
55-63,	2.60	1.71	3.84	7.92
64-72,	3.47	4.00	2.28	8.34
Total,	27.95	50.46	44.38	108.81

The results given in column I are from a field left for 30 years unmanured and exhausted by continuous cropping to beans, followed by fallowing. In column II it is seen how, even under condition of the most heavy drain upon a soil, the supply of available nitrogen can be maintained by the use of stable manure.

In columns I and II above, the comparatively large quantities of nitrates in the lower zones of the soil will be noted as indicative of the effect of excessive downward percolation during 4-5 years of fallowing; for this reason the soils of the Geescroft field are really poorer in available nitrogen, within that zone occupied by the bulk

of the roots, than the amount of total nitrogen to a depth of 72 inches would indicate.

In column III the effect of 35 years' continuous culture of wheat on the same land is shown; the result is a soil richer in available nitrogen than might be expected.

The comparative effect of 17 years in clover, as shown in the last column, is a marked increase of available nitrogen, and shows the good effect of such crops in increasing the store of this important element of plant food.

The effect of permanent grass in increasing the store of nitrogen in the soil is marked, and is well brought out in the following table by Sir. J. B. Lawes:³⁵

Table XI.

Nitrogen in Surface Soil (dry), First 9 Inches, and Gains in Pounds Per Acre in Land in Permanent Grass.

Dates.	Number of years.	Nitrogen.	Gain.	
		Per acre—total lbs.	Per acre—total.	Per acre—per annum.
1856,	3,040
1866,	10	3,497
1879,	13	4,091	457
1888,	10	4,690	594	45.7
			599	59.9
Total,	33		1,650	50.0

It should be understood that the above field has been mowed for hay every year for 33 years, with average yield of 1.7 tons per acre per annum, and yet, notwithstanding this annual drain, there was an increase of nitrogen in the soil of 50 pounds per acre per annum.

Thus the state of knowledge is sufficient to indicate that all soils can be kept sufficiently rich in available nitrogen by the judicious use of leguminous crops in a proper system of rotation, or by the use of grass and clover as a part of the same system, and this without the necessity of purchasing a single pound of nitrogen in a fertilizer.

6. Denitrification and Loss of Free Nitrogen.

Through the agency of bacteria present in all soils, nitrates under certain conditions may be converted into lower oxides of nitrogen, into ammonia or into free nitrogen.

Goppelsröder,⁵⁶ in 1862, made the observation that in soils rich in humus active denitrification took place.

In 1882, Gayon and Dupetit⁵⁷ found that in river water containing small quantities of nitrate of potash (0.02-0.2 grams per 1000) there was a reduction of the latter salt to ammonia.

The reduction of nitrates through the agency of bacteria was later (1883) observed by Dehérain and Maquenne,⁵⁸ and also by Springer,⁵⁹ which reduction they held to be due to the agency of anaerobic forms, similar to *B. butyricus*, which either reduced the nitrates to lower oxides of nitrogen or to free nitrogen.

Heraeus,⁶⁰ in 1886, isolated from water two bacilli which possessed to an eminent degree the power of reducing nitrates to nitrites. Blasi and Fravoli,⁶¹ in 1888, found in Palermo soil 27 different species, which they have studied as to their chemical action in gelatin containing nitrates. They found that in 1-3 days the quantities of nitrates diminished with a simultaneous increase of nitrites. These latter reached their maximum in 6-8 days, and after 25-30 days entirely disappeared.

Frankland,⁶² in 1888, isolated from water some 32 different species of bacteria, of which no less than 17 possessed the power more or less completely of reducing nitrates to nitrites. Of these the most strongly reducing were *B. ramosus* and *B. pestifer*.

Bréal,⁶³ in 1892, isolated from straw and other refuse a ferment which possessed strong reducing action. He found that if to straw fermenting in water, nitrates were added, the latter rapidly disappeared, while if sterilized straw were put into water and allowed to ferment, no such reduction took place, thus showing the presence upon the straw of some specific denitrifying organism. The nitrogen, according to the author, appeared partly in organic combination and partly as elementary nitrogen.

Gilthay and Aberson,⁶⁴ in 1892, isolated from both soil and atmosphere two organisms which possessed active powers in reducing nitrates, and which they named *Bacillus denitrificans* var. a. and b. Both of these liberated free nitrogen.

Egunow,⁶⁵ in 1893, isolated from the surface of seed a bacillus which possessed the power of reducing nitrates to nitrites, etc. Egunow found that in flasks with broad flat bottoms, with mineral media and nitrates, and with the fluid only a few millimetres thick, the nitrates were finally converted into ammonia. Where the thickness of the fluid was 10 mm., the nitrates were converted into am-

monia and free nitrogen. Where the thickness was 60-70 mm. no ammonia was formed, but only free nitrogen.

Burri, Herfeldt and Stutzer,⁶⁶ in 1895, isolated from horse manure and from straw two bacilli, respectively *B. nitrificans* I and II, which actively reduced nitrates.

Schirokikh,⁶⁷ in 1893, isolated from horse dung a bacillus which liquified gelatin, and actively reduced nitrates. In broth containing 2.5 grams of potassium nitrate to the litre, the latter was completely reduced in 5.8 days. at a temperature of 30-35 degrees C.

Again, Sewerin,⁶⁸ in 1897, isolated from horse manure 29 species of bacteria, of which 20 possessed greater or less power of reducing nitrates.

In 1896, Richards and Rolfs⁶⁹ conducted some experiments with 25 different solutions prepared to typify conditions of water polluted with decaying organic matter (sewage), and at the same time containing nitrates.

They note, (1) the rapid disappearance of nitrates, usually less than 10 per cent. of the original quantity remaining at the end of 3 days; (2) a corresponding increase of nitrites; (3) that when the solutions contained no organic matter other than that usually present in the water reduction took place very slowly and incompletely; (4) that the nitrogen which disappeared from the nitrates was finally given off in the free state; (5) that whenever nitrates were added to decomposing organic matter under such conditions that the growth of the bacteria required more oxygen than the solution afforded, the latter took it from the nitrates setting free nitrites, which in time were decomposed, setting free nitrogen.

From the foregoing citations it is seen that denitrifying bacteria are abundantly distributed in nature, and that they are found in water, soil, manure, sewage and upon the surface of plants, particularly upon straw. It is only necessary to add a small portion of soil to media containing nitrates to obtain active denitrification thus showing the general presence of denitrifying bacteria in soils.

The majority of soil bacteria studied separately by the author possess this property to a greater or less degree.

7. Conditions Affecting Denitrification.

(a.) *Presence or Absence of Air*.—It has commonly been supposed that the power of reducing nitrates belongs more exclusively to the anaerobic bacteria, or those which live without air. The question is an open one as to how far denitrification is the result of a deficient supply of atmospheric air. All of the species of soil bacteria so far

examined by the writer, with one exception, are aerobic, and yet they all actively reduce nitrates.

That active denitrification can take place in the presence of an abundant supply of atmospheric oxygen is shown by the following: A culture of *B. pulvinatus* was selected on account of its active denitrifying properties.

The bacillus was grown in a solution of Witte's peptone containing one gram of nitrate of soda to the litre.

Provisions were made for continually passing sterile air through the culture so as to provide an abundance of atmospheric oxygen. Simultaneously with the above, a culture was made in an ordinary flask, plugged with cotton wool, without aeration. At the end of 5 days 7.0 milligrams of nitrite of soda per 100 c. c. were found in the aerated culture and 30.0 milligrams in the non-aerated.

At the end of 10 days 20 milligrams of nitrite of soda were found in the aerated and 40 milligrams in the non-aerated culture.

In this case active denitrification took place even with abundant and continual aeration of the culture, although the presence of large quantities of atmospheric oxygen seemed to somewhat retard the process.

These results are in conformity with those of Stutzer and Maul.⁷⁰ *B. denitrificans* and *B. coli-communis* in a broth culture caused a vigorous reduction of nitrates to nitrites, and in four days the nitrites had entirely disappeared, when, however, a constant stream of air was passed through the culture, growth took place as before but the nitrates had not entirely disappeared until after the tenth day.

It would, therefore, appear that denitrification can take place actively even in the presence of an abundant supply of atmospheric oxygen, certain bacteria at least being capable of utilizing combined oxygen equally with that supplied in the free state.

Contrary results were obtained by Pfeiffer, Franke, Götze and Thurmann⁷¹ in their study of the loss of nitrogen in manures. They found that denitrification was more active when air was drawn through and over the manure than when air was excluded, the presence of atmospheric air apparently favoring the process, and it has become a recognized principle that manures lose nitrogen less readily when kept closely compacted than when loose.

(b.) The Presence of Organic Matter.

The effect of organic matter upon the development of the denitrifying organism was shown by Stutzer and Jensen in 1897.⁷² The experiments of the latter indicate that denitrification can take place only in the presence of a sufficient supply of assimilable carbon.

otherwise the nitrates remain unaltered although the denitrifying organism may be present in abundance. Apropos to this principle the authors hold with Maercker that horse manure is more active in causing denitrification than sheep or cow manure since the former is much richer in assimilable carbon than the latter.

It is a well known fact that denitrification is particularly active in stable manure, and denitrifying bacteria are especially abundant therein. The admixture of straw also favors the denitrification process, and an explanation of this has been found in the fact that straw contains an easily assimilable carbon in the form of pentosans. The pentosans which are abundant in coarse manure and straw, furnish a much more readily available food to denitrifying organisms than cellulose or fibre. Still more readily assimilable forms of carbon are found in such compounds as glycerin, citric acid, lactic acid, etc., and Pfeiffer and Lemmermann found that the denitrification process in manure was accelerated by the addition thereto of a soluble calcium citrate.

The loss of nitrogen in the free state in organic infusions seems to be associated with the presence of readily decomposable nitrogenous bodies, such as the *amides*. Thus Grimbert^{72,5} found that the colon bacillus, when grown in a solution containing peptones and nitrates, yielded no free nitrogen, but when grown with nitrates in a solution made from beef extract and containing amides there was a considerable loss of nitrogen in the free state.

The author, therefore, concludes that the nitrogen does not come exclusively from the nitrates, but also results from the denitrifying action of the bacillus upon amido principles in the culture medium.

2. That the evolution of free nitrogen seems to result from the secondary reaction which nitrous acid, formed by the bacteria, exerts on these amido substances.

It has been seen that one step in putrefaction and ammonification is the production of amido acids and basic amines, and this explains perhaps the rapidity of the denitrifying process in infusions rich in nitrogenous organic matter which are undergoing putrefaction.

Kinger⁷³ showed that when nitrate of soda was mixed with liquid manure (urine) there was a decided decomposition of nitrogenous compounds and loss of nitrogen. And in the same connection, T. B. Wood⁷⁴ found that nitrate of soda when used alone as a fertilizer for oats gave much better results than when used with manure.

This simply reiterates the former principle that nitrates readily decompose in the presence of an excess of readily assimilable organic matter.

(c.) The Relation of Denitrification to Cultivation.

It has been stated that denitrification may take place either in the presence or absence of atmospheric air.

With the presence of some form of easily assimiliable organic matter, it may take place under any condition of aeration.

Nitrification is the opposite and antagonistic process to denitrification. Furthermore, the conditions which are favorable to the former are inimicable to the latter.

The question of denitrification and loss of nitrogen in soils is one about which much has yet to be learned, but it is generally believed that under ordinary conditions it is of no particular importance. At least this much may be said, that if the agriculturist will maintain those conditions which are favorable to nitrification, any possible loss of nitrogen by the opposite process can be disregarded.

Hence cultivation, which effects the rapid destruction of easily assimiliable organic compounds, leaves little opportunity for the denitrifying bacteria to carry on their destructive work.



8. The Loss of Nitrogen From Stable Manures and its Conservation.

It has been stated that denitrification and loss of nitrogen is comparatively rapid in stable manures, because of the abundant presence of denitrifying bacteria and of easily assimiliable organic compounds which furnish food to the latter. It has also been noted that straw mixed with urine and excrement also assists the process by furnishing an easily assimiliable carbon compound in the form of pentosans.

So deleterious is the action of straw that it has been suggested, as a feature of good farm practice, to keep the manure and litter separate, or to see to it that as little straw as possible becomes mixed with the urine and faeces.

To what extent this is practicable must be left to the practical agriculturist to determine. But taking conditions as they ordinarily exist, i. e., with straw forming a considerable proportion of the manure proper, and with the natural losses of nitrogen which must take place to confront us, how can these be reduced to the minimum.

There are two methods for conserving the nitrogen content of stable manure, first, by the exclusion of air and second, by the use of preservatives.

Regarding the first method it has been shown that denitrification is more active when air was drawn through and over the manure than when it was excluded.

Under the former condition of free access of air Franke, Götze and Thurman⁷¹ showed a loss of nitrogen amounting to from 27.6 to 42.6 per cent. of that originally present.

Hence to prevent loss of nitrogen the manure should be kept well compacted; its storage in sheds, or even in closed receptacles, whereby it is protected from strong currents of air, is also advisable.

The preceding authors have also made valuable experiments on the use of preservatives. Of these, the use of super-phosphate is especially recommended.

In this case the transformation of the nitrogen in the form of free ammonia into free nitrogen was prevented by the use of a sufficient amount of the super-phosphate to combine with all the ammonia formed, thus fixing it and preventing its loss. Plaster or sulphate of lime acts in the same way but less energetically.

The addition of 2-3 per cent. of caustic lime, or 5 per cent. of marl, decidedly reduced the denitrifying power of fresh manure.

The use of caustic lime, however, is not to be advised as it was found to promote ammoniacal fermentation, and the loss of ammonia.

According to the authors, the mechanical condition of the manure exerted a more marked effect upon its preservation than chemical preservation. Hence the keeping of the manure in a well compacted condition and free from strong currents of air is of primary consequence.

9. The Disintegration and Dissolution of Mineral Matter.

This subject has, in a measure, been discussed in previous pages, but a few additional statements, in this connection, might be made.

The mineral elements of plant food are absorbed in the form of salts. These salts are compounds of acids and bases, as shown in the following list:

Plants Absorb.

Acids.	Bases.	As Salts—i. e.	
Nitric,	Ammonia,	Nitrates,	} of { Ammonia. Potash. Lime. Iron. Soda or Magnesia.
Sulphuric,	Potash,	Sulphates,	
Carbonic,	Lime,	Carbonates,	
Phosphoric,	Iron,	Phosphates,	
Silicic,	Soda,	Silicates or	
Hydrochloric,	Magnesia,	Chlorides,	

Of these acids, certain of them have existed in the rocky crust of the earth, from which soils have originated, before the advent of life, and hence are not of bacterial origin. These are sulphuric, phosphoric, silicic and hydrochloric acids. Others of them are products of bacterial life such as carbonic and nitric acids.

Of the bases, all except ammonia are of primordial or earth crust origin.

Of the salts, the sulphates, silicates, carbonates and chlorides, which are largely present in rocks, are not absorbed except in minute quantities, the greater part of the bases being taken up as nitrates and phosphates, and also as salts of the organic acids.

In the process of nitrification the nitric acid combines with the various bases present in the soil, and nitrates are produced.

Phosphoric acid exists in the soil in the form of insoluble basic phosphates, which, under the action of organic acids, are converted into neutral or acid salts which are soluble. Hence the production of organic acids by bacterial fermentation renders phosphoric acid available to plant roots.

We have already spoken of the action of carbonic acid, in disintegrating, and setting free as carbonates, the various bases locked up as mineral silicates. These carbonates unite with silica to form zeolites and these in turn are slowly decomposed by organic acids, and their contained bases again liberated as organic salts.

H. Carrington Bolton has shown that many minerals are slowly decomposed by the action of cold citric acid, the zeolites and other hydrous silicates being especially susceptible.

Thus by the combined production of carbonic, nitric and the various organic acids, through the action of bacterial life, we have all the necessary agencies at hand for the dissolution of the mineral elements of plant growth.

VIII. THE ASSIMILATION OF ATMOSPHERIC NITROGEN.

1. HISTORICAL SUMMARY.

The Biology of Root Tubercles.—It is a fact familiar to all that the roots of leguminous plants contain nodular swellings or excrescences known as "root tubercles."

These have been recognized from the earlier days of botanical inquiry, and as far back as 1615, DeLéchamp used the characters of the root tubercles as a basis of classification, and the same use was made of them by DeCandolle⁷⁵ in his *System of Vegetation*.

At different periods during the earlier part of the nineteenth cen-

tury various opinions were held by different authors regarding these bodies. The more general view was that they were of the nature of fungus growths, or otherwise diseased conditions of the roots; a few held them to be the result of animal parasitism, and due either to insects or worms; while others considered them to be normal organs, regarding whose function there was difference of opinion.

All of these latter views were, however, purely speculative, rather than based upon any serious study; and it was not until 1866 that any attempt was made to study them closely; when Woronin⁷⁶ described their microscopic structure.

He found them to be composed of a central portion of thin walled cells, of an outer rind, and of an intermediate layer or vascular ring. In the central portion the contents were cloudy, and closer examination showed the cells in this portion to be filled with peculiarly shaped bodies, which were sometimes rod-like, at others forked, and presenting a variety of forms simulating the letters T and Y. Woronin regarded these bodies as living organisms, but since they differed from other bacteria or *Vibrios* in form, he termed them *Bacteroids*. He regarded them as the causative agents in the formation of the tubercles, although he offered no proof of this assumption.

In 1874, Erickson⁷⁷ found in the newly developing tubercles long branching filaments resembling the mycelium of a fungus. These threads he considered to be the infecting agents. Later on in the development of the tubercles he observed the presence of the bacteroids, noted by Woronin, but failed to connect the two as correlated structures.

In the early observations of Woronin and Erickson we have the germ and substance of all that has since been discovered regarding root tubercles. Both recognized, as is held to-day, the two classes of bodies the filamentous and the bacteroid. Both were right in assuming that the organisms as seen within the tubercles were the agents in their production, although Erickson was more nearly right in assuming that the filaments were the real infecting agents.

But it was left to others to show the relation of the filaments to the bacteroid bodies, and this question became the subject of much controversy and difference of opinion extending over many years. In the controversy which followed, covering the latter 25 years of the nineteenth century, the most eminent botanists were engaged.

On certain points they agreed, on others they differed, but since the points on which they differed determined the position which these forms should occupy in a system of classification, the subject became of especial interest.

All agreed to the presence of the filaments within the tubercles in the early stage of their development. These filaments were fur-

thermore regarded as the infecting parts, which forced their way into the younger roots and, by their irritating presence, set up a multiplication or proliferation of cells which resulted in the building up of an excrescence or tubercle.

It was upon the structure of the filaments, and upon how the bacteroid bodies originated from them, that they differed, and upon this point it is surprising that there should have been such a wide diversity of view. These two views class themselves under two heads, which, for the sake of convenience, we shall term the endogenous and the exogenous theories. The former was held by Prazmowski, Frank and Maria Dawson, and the latter by Laurent, Ward and Atkinson.

According to Prazmowski,⁷⁸ the leader of the endogenous theory, the infecting agent in the production of the root tubercles is present in the soil as a definite bacterium, which, after Beyerinck, he calls *Bacterium radicola*.

Certain of these force their way into the smaller roots and for a time multiply therein; but soon the juices of the plant exert an unfavorable influence upon them, and to protect themselves against its injurious action they excrete a gelatinous substance in which they remain embedded, and in which they continue to multiply. With this multiplication the gelatinous envelope is drawn out into long strands or filaments, containing the rod-shaped bodies or bacteria. As long as these remain protected by their gelatinous envelope they continue to multiply and retain the rod-shaped form.

As the filaments approach the central portion of the tubercle they swell out into rounded vessicles. Later on by the enlargement of these latter the enveloping membrane becomes so thin that it bursts and the enclosed bacteria are set free. From this time they cease to multiply, and by degeneration assume a variety of irregular branched forms, first observed by Woronin, and called by him bacteroids. Prazmowski, therefore, recognized (1) that the filaments were elongated pouches of gelatinous matter, the secretion of the bacteria, and in which the infecting bacteria were embedded; (2) that these bacteria multiplied as straight rods within the filaments, or endogenously; (3) that by the escape of the bacteria they underwent various degenerations by which they assumed the irregular forms of bacteroids.

One essential point in Prazmowski's position was that the bacteroids were degenerated forms.

Frank⁷⁹ in his later observation, held to the position of Prazmowski, but differed in regarding the gelatinous envelopes as a product of the cell rather than of the bacteria.

Maria Dawson⁸⁰ in 1899, also held to the view of Prazmowski. By proper staining she found the filaments to consist of strands of

straight rodlets lying parallel to the longer axis of the filaments and embedded in a colorless matrix. This matrix did not consist of cellulose, chitin, or any form of slime. The swellings, or vessicles of Prazmowski, occurred at places where the rodlets had become heaped up and eventually had burst liberating the rodlets. After liberation from the filaments they became transformed into X, V and Y shaped bacteroids.

The exogenous theory is best represented by the researches of Laurent in 1891,⁸¹ and later by those of Atkinson in 1893.⁸²

Both hold that the filaments are not pouches containing bacteria, but true homogenous filaments, which, as they enter the cells of the central portion of the tubercle, show enlargements which afterwards, by a process of budding, give rise to the branched bacteroid bodies.

This view was also held by Ward⁸³ somewhat earlier, and all agree in regarding the organism as a lowly organized fungus instead of a bacterium whose filamentous portion corresponds to the mycelium, and whose bacteroids are buds or gonidia thrown off from the latter. For this fungus, Laurent adopts the name *Rhizobium leguminosarum* originally proposed by Frank.

Regarding these two views the bulk of evidence inclines to the exogenous theory, although there is opportunity for further study before the matter is finally settled.

In what has been already said it was assumed that the root tubercles of leguminous plants are caused by an organism, commonly present in the soil, which infects the younger roots and results in the formation of excrescences or tubercles.

In the earlier researches on the structure of the tubercle and their contained organism it was assumed that the associated fungus was the cause of the tubercle. But the first demonstrable proof of the relation of soil organisms to the development of tubercles was due almost simultaneously to Hellriegel⁸⁴ and to Ward,⁸⁵ in 1886.

Hellriegel grew peas in sterilized sand free from nitrogen, but to which the other mineral fertilizers had been added. The plants grew until the stock of nitrogen in the seed was exhausted, when they showed signs of starvation. To these starving plants were added infusions of soil in which peas had previously been grown; in one case this infusion was sterilized, and in the other not. When unsterilized soil infusion was used, the plants began to revive their vigor, and continued to grow to maturity. On their roots numerous tubercles developed.

In the case of the plants watered with sterilized soil infusion, the plants did not revive, and no tubercles formed.

From this experiment and others of like character it was shown that something, probably a living organism, existed in the soil in-

fusion, which was capable of infecting the roots and causing the formation of tubercles; and that by heat this organism could be destroyed.

The experiment also showed that the renewal of vigor of the plant in a nitrogen free soil was correlated with the infection of the roots and the formation of tubercles; in other words, tubercle formation and nitrogen assimilation by the plant were interdependent.

From this point the question of tubercle formation passed from one of mere botanical interest to one of great agricultural importance.

Ward⁸⁵ went even further, and besides showing that tubercles could be produced at will by the inoculation of roots with soil infusions, succeeded in tracing the development of the fungus filaments into the root hairs, and thence into the cortex of the root, where he noted the development of the tubercles at these points of infection.

In 1888,⁸⁶ Beyerinck, succeeded in cultivating the root tubercle organism on artificial media, and described a number of races from different plants, which he considers varieties of one species of bacteria and which he names *Bacillus radicicola*.

In 1890, Prazmowski,⁷⁸ by cultivating the organism of the root tubercles of beans, succeeded in inoculating their roots with pure cultures of the latter by watering sterilized soils in which the plants were grown, with liquid cultures of the organism.

In 1891, Laurent,⁸¹ by growing legumens in water culture, succeeded in inoculating their roots and producing tubercles at the points of inoculation by puncturing them with a needle whose point was contaminated with root tubercle germs. And a little later, Atkinson⁸² succeeded, by growing vetch in water culture, in inoculating their roots and producing tubercles from pure cultures of the organism.

There is thus no doubt but that tubercles on the roots of leguminous plants are produced by infection from without, and by an organism entering the root from the soil.

The organism as it exists in the soil is probably in the form of bacteria-like bodies, and most soils contain them in greater or less abundance. Their method of infecting the root is best described and figured by Atkinson.⁸²

The bacterial body has the power to penetrate a root hair and is first seen therein as a filament, extending its entire length, as shown in Fig. 7 B-C, and thence entering the cells of the cortex of the root, causes their proliferation and the production of a swelling upon the side of the root, Fig. 7 A, which by further growth becomes a tubercle. Within the central cells of this tubercle the filaments can be seen branching in all directions as seen in Fig. 8 A.

At numerous points are seen irregular swellings, Fig. 8 A-B,

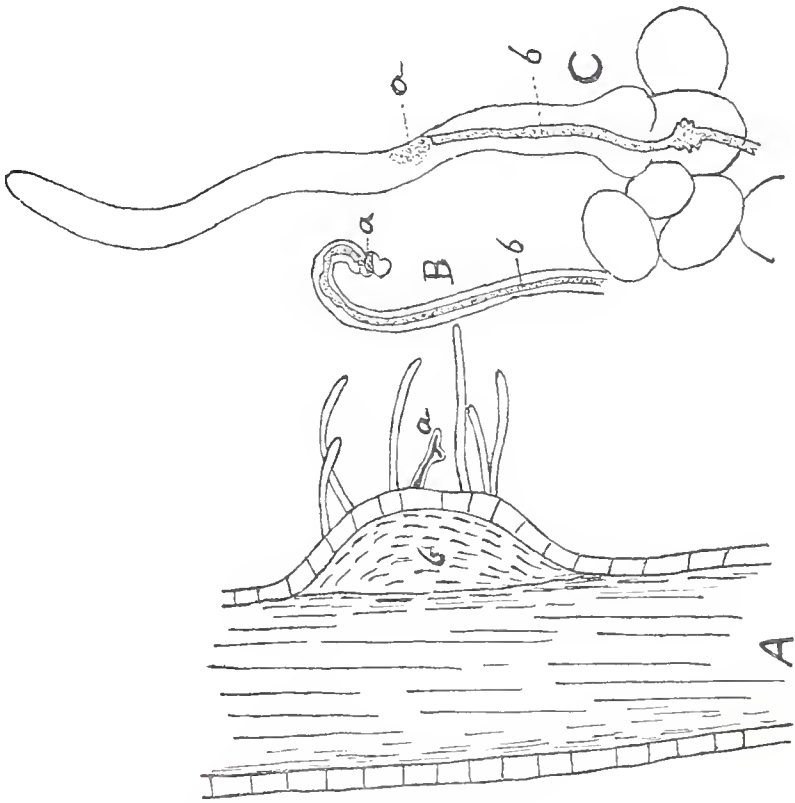


FIG. 7.—Root tubercle formation. Infection of root through root hairs. A, a, infected root hair; b, beginning of a tubercle; B, a, point of entrance of infecting filament into root hair; b, infecting filament; Ca-Cb, as before. (After Atkinson.)

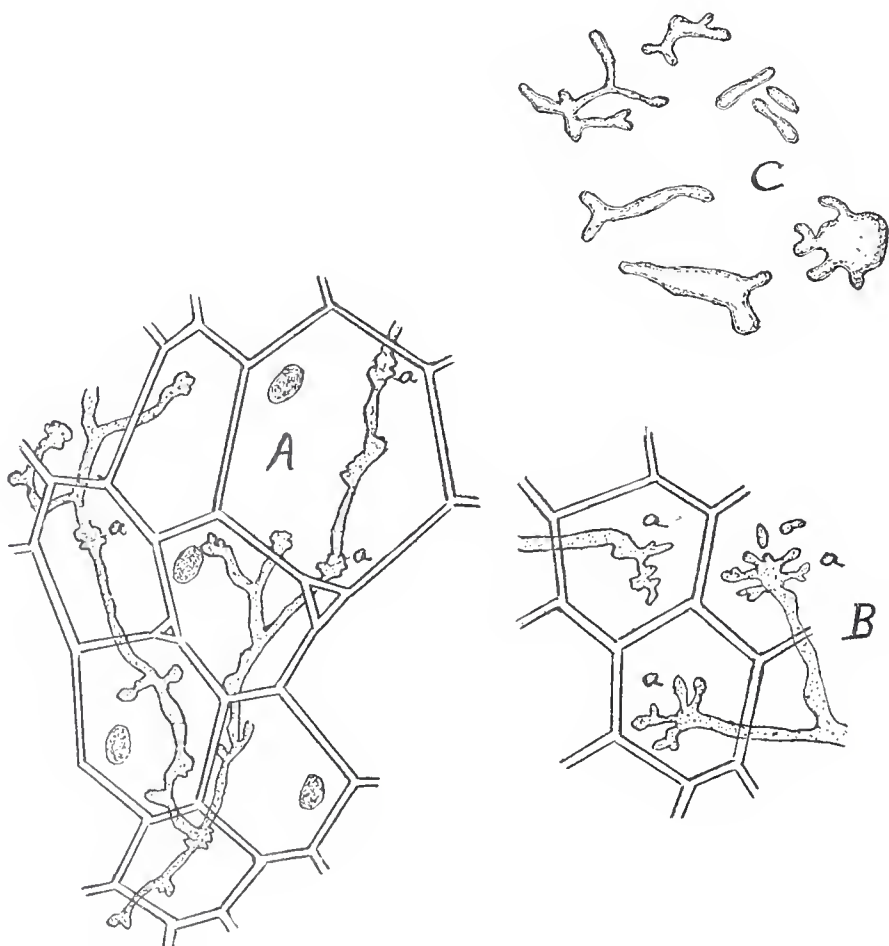


FIG. 8.—Root tubercle formation. A, ramification of filaments within central cells of tubercle; a a, enlargements of the latter from which buds originate. B—aa, showing budding of filaments and production of bacteroids. C, bacilliary and bacteroid bodies. (After Laurent.)

which later on produce buds which become bacterial and bacillary bodies, and which eventually fill the cells of the central portion of the tubercle.

Such in brief is an outline of the biology of the root tubercle organism. For a further exposition of the subject the reader should consult the excellent paper of Atkinson.

2. THE RELATION OF ROOT TUBERCLES TO NITROGEN ASSIMILATION.

It has long been known that clovers enrich land, but the full philosophy of the matter has not been fully understood until comparatively recent date.

Lachmann,⁸⁶ in 1858, was probably the first to suspect that certain nodular bodies seen upon the roots of legumes were in some way connected with the absorption of nitrogen by the plant, but no experimental proof of this assumption was given.

In 1864, Rautenberg and Kuehn⁸⁷ gave the first inkling of this in their work on water cultures. They noticed that with certain legumens grown in water, nodules formed on the roots of only those plants which were growing in a solution free from some compound of nitrogen. The presence of nodules, therefore, seemed to be associated with an effort on the part of the plant to obtain nitrogenous food.

DeVries,⁸⁸ a little earlier, observed that comparatively few nodules developed on plants to which an abundance of nitric nitrogen was supplied. Root tubercle development, therefore, seemed to be associated with nitrogen hunger. But an exact demonstration of the relation of root tubercles to nitrogen assimilation by the plant was not made until 1886, when Hellriegel announced his important series of researches.

Hellriegel⁸⁴ was able to grow peas and other legumens in pure sand absolutely devoid of nitrogen, but supplied with other plant food. The sand was sterilized so as to destroy all germ life therein. Peas etc., grew in such a soil only under certain conditions, i. e., when the soil was infected with an infusion containing the living germs of root tubercles. Soils inoculated with sterilized infusions, or soils not inoculated and previously sterile, failed to grow peas.

In the infected soils the peas developed tubercles, and with their development the plants thrived and attained their full maturity. Since the soil was devoid of nitrogen, the plants could have gained the nitrogen for their fruition from but one source, and that was the atmosphere.

Hellriegel thus, as a result of an elaborate series of experiments, was able to announce positively that leguminous plants were able to utilize the free nitrogen of the air.

But an announcement so radical and startling as this could not rest on the authority of one man alone, and accordingly, Hellriegel's experiments, with modifications, were repeated by numerous investigators, notably, Nobbe, Hiltner and Schmid,⁸⁹ with the same results.

In Hellriegel's experiments his plants were grown in the open air, and since atmospheric air contains combined nitrogen in the form of ammonia and nitrous acid, it was a question, though the soil might be free from nitrogen, whether the nitrogen compounds present in the air might not be the source of nitrogen to the plant instead of free nitrogen.

Accordingly, in 1892, Atwater and Woods,⁹⁰ in this country, grew peas in glass cases the air of which was free from every trace of combined nitrogen. The soil and nutrient solution used to water and fertilize the plants were also nitrogen free. At the end of the experiment, covering 85 days, 27 plants had made an average growth of 29 inches, with a total gain of 242 mg. of nitrogen. It was furthermore noted, that as a rule the largest gains were in those plants which showed the largest tubercle development. Thus, the authors conclude, "The free nitrogen of the air was alone available to the plants, and the gain must have been by the acquisition of free nitrogen."

There is, therefore, no longer any doubt remaining in the minds of scientists that leguminous plants possess the power of utilizing the free nitrogen of the atmosphere.

It has also been shown that they do this in proportion to the poverty of the soil in available nitrogen. In other words, the plant does not utilize the function of appropriating free nitrogen unless forced to do so.

A legumen will thrive in the presence of an abundance of available nitrogen in the soil, or will respond to a liberal application of nitrate of soda. And it does so because it is easier for it to utilize a form of nitrogenous plant food already prepared for it than to go through the more elaborate process of appropriating the free nitrogen of the air.

But if the soil be deficient in nitrogenous food the legumens will extend their appropriating energies along these new lines, eventually overcome their soil environment and come off the victor.

On this principle is based the value of leguminous plants as soil enrichers.

It has been stated, and has perhaps been fairly well established, that the increase in nitrogen in legumes is proportionate to the development of tubercles.

In his experiment with beans, Halsted⁹¹ found that in every case there was a decided increase in yield of beans on soils where several successive crops of beans had been grown, over the yield where

grown on a soil for the first time. The roots of the plants growing upon old ground were well supplied with tubercles, while in the new soil they were usually almost entirely absent.

Lane,⁹² in New Jersey, obtained similar results with the cowpea, when grown for three successive crops on the same land. The first season but few tubercles were noted, and the yield was 6.56 tons per acre. The second year the tubercles were more abundant and the yield was 7.19 tons per acre. The third season the tubercles grew abundantly and the yield per acre was 10.02 tons.

The fertilizer applied the third season was less than one-half that applied the second and it is believed that the increase was due in a large measure to a greater abundance of tubercles.

It is a general observation that both annual and perennial legumes grow better a second season than they do the first, and this is probably due to a larger seeding of the soil with the organisms necessary to the production of tubercles on the roots.

It is claimed by Nobbe and others that root tubercles exert no influence in nitrogen assimilation when there is an abundance of available nitrogen in the soil. The tubercles being held to be the organs for such assimilation, this amounts only to saying that these organs will not perform this function unless called upon to do so, but let this necessity be forced upon the plant, the function of the tubercles is exercised, and the greater their number the greater will be the gain of the plant in nitrogen.

Furthermore, the poorer the soil in available nitrogen, as shown by Hiltner⁹³ in the case of *Alnus glutinosa*, the greater is the number of root tubercles which will develop, provided the necessary organisms are present in the soil. Hence under conditions of nitrogen starvation or deficiency the number of tubercles on the roots becomes a measure of the nitrogen assimilating activities of the plant.

3. DO OTHER PLANTS THAN LEGUMES ASSIMILATE FREE NITROGEN?

The power of legumes to assimilate free nitrogen does not rest on the fact that they belong to a certain family of plants, the Leguminosae, but rather to the fact that their roots contain tubercles. This is instanced by the common alder whose roots bear tubercles. Hiltner has shown that young alders without root tubercles, and deprived of combined nitrogen, are unable to assimilate atmospheric nitrogen, and that the plants are poorly developed. But when by inoculation, tubercles are produced, the plant can utilize free nitrogen as in the case of legumes. This is well illustrated in Fig. 9.

The alder is therefore the one non-leguminous plant which is able to assimilate atmospheric nitrogen.

Regarding other non-leguminous plants, the question of nitrogen assimilation has been raised, but the answer has, on the best authority, been in the negative. Thus Lotsy⁹⁴ showed the inability of white mustard to utilize free nitrogen and the same was shown by Nobbe and Hiltner⁹⁵ regarding mustard and buckwheat.

So far as present knowledge goes we may, therefore, assume that plants without tubercles are unable to utilize free atmospheric nitrogen.

4. ARE THERE DIFFERENT SPECIES OF ROOT TUBERCLE ORGANISMS.

This is a question which has frequently been raised, but which as yet cannot be positively answered.

Nobbe held that there is a separate race of bacteria for each species or group of nearly allied species of plants. On the other hand Mazé, an equally good authority, claims that there are certain physiological forms of bacteria determined by the nature of the media in which they are developed. These are able to inoculate the roots of plants growing in soils offering the proper conditions for their development.

Beyerinck⁹⁶ has made a careful study of the different forms of bacteroids found in different species of legumens, and records differences among them. Voelcker⁹⁷ illustrates 16 different forms of bacteroids from different species of legumens, but states that on cultivating these organisms on nutrient media their differences disappear and that they can no longer be distinguished the one from the other.

This would indicate that differences in the form of the organism, as seen within the plant, has little significance, and is controlled perhaps entirely by the differences in the host. For it is a well known fact that one and the same bacterial organism will often present variations in form when grown in media of different character.

It is thus evident that the question of differences of species cannot be settled from the standpoint of differences of form, but must be determined from the physiological side.

Nobbe, Hiltner and Schmid⁹⁸ have thrown important light on the question in their inoculations of different legumens with pure cultures.

Their experiments show that the organism from a single species of legumes is capable to a greater or less degree of infecting and of producing tubercles on the roots of a number of distinct species. Thus the vetch can be infected by means of culture from Robinia (locust) Acacia, Vicia (vetch), and Pisum (pea); but the greatest de-



FIG. 9.—*Alnus* in sterilized soils. Pot 1, inoculated with bacteria from *Alnus* tubercles. Pot 2, soil not inoculated. (After Hiltner.)



FIG 10.—*Robinia pseudacacia* in nitrogen free soils. Pot 1, inoculated with bacteria from pea tubercles. Pot 2, inoculated with bacteria from *Robinia* tubercles. (After Nobbe, Hiltner and Schmid.)

velopment of the vetch took place when its roots were inoculated with vetch organisms. In other words, a given legumen is most susceptible to organisms of its own kind, and but feebly so to those of another species. (See Fig. 10.)

In the following table are given the results of these inoculations by the above authors:

Chemical Analysis of Plants.

	Inoculated With Pure Cultures From			
	Robinia.	Acacia.	Vicia.	Plum.
Robinia dry substance—grams,	7.402	1.158	0.858	1.479
Robinia nitrogen—milligrams,	232.100	16.600	13.500	21.100
Acacia dry substance—grams,	1.953	6.943	1.248	1.817
Acacia nitrogen—milligrams,	17.000	109.800	16.200	19.700
Vicia dry substance—grams,	0.783	0.866	9.133	1.033
Vicia nitrogen—milligrams,	12.900	14.700	264.000	22.600

The differences here noted can only be explained by a consideration of the question of virulence of the organism, and of specific adaptation.

The pea tubercle organism by its growth in the pea root becomes especially adapted to that host, and losses its adaptability to another host. This, however, is a property more or less elastic, and subject to artificial modification.

Thus Nobbe and Hiltner ⁹⁹ have shown that when peas were inoculated with cultures from bean tubercles, some tubercles would be formed but the organism seemed to be without the power of assimilating nitrogen; but if the same inoculation was continued upon a second generation of plants, the bacteria became nearly as efficient as those from the roots of the same genus. In other words, by passing pea tubercle bacteria successively through beans, the virulence at first feeble was decidedly increased.

The same authors have also shown that different cultures possess different powers in producing tubercles. Thus if a plant already possessing tubercles is inoculated with a culture of the same virulence there is no increase in the number of tubercles, but when inoculated with a culture of a higher virulence there is an increase in the number and size. The authors, therefore, hold that a plant may

acquire a certain immunity against infection by bacteria of a certain degree of virulence.

This may account for the failure of land in many instances to grow clover, so called "Clover sickness," when there is reason to believe that the soil is well supplied with the specific organisms.

To return to the question of species among different root tubercle organisms, it may be concluded that distinct species do not exist, and that there is but one species of variable virulence, capable of infecting the roots of any of the legumens either feebly or readily. That this species, which may at first attack a given host but feebly, has its virulence so increased by repeated growth in that host that it eventually is able to produce ready infection.

5. RELATION OF THE SOIL TO TUBERCLE PRODUCTION.

It has been stated that legumes will grow readily in soils where there is an abundance of available nitrogenous food independent of their power to assimilate atmospheric nitrogen. The legumes, however, are so rich in nitrogen, and such vigorous growers as a rule, that their demands for nitrogen are greater than those of any other class of agricultural plants. Hence, in ordinary soils, legumes must utilize their nitrogen assimilating function to a greater or less degree.

In poor soils, and these are the ones in which we desire to grow legumes as soil enrichers, there is often at first a struggle on the part of the legumes for existence, particularly is this true when a new legume is introduced.

It is assumed that in most soils root tubercle organisms of one variety or another exist, but often in such few numbers or in such an attenuated form as to feebly affect the plant. In this event an introductory crop of that legume, however small, may be necessary for the purpose of stocking the soil not only with the necessary number of organisms but with those of the proper degree of virulence. Soils, therefore, become adapted to the growth of any legume and this adaptation consists in an abundant supply of organisms of the proper virulence to infect the roots.

Nothing directly is known regarding the relation of soil acidity to the life of root tubercle bacteria therein, but Salfeld found that the addition of lime to land was beneficial to the development of root tubercles on field peas, lentils and garden peas.

6. INOCULATIONS OF SOILS.

As soon as it was shown that root tubercle development was dependent upon the presence in the soil of specific organisms, the question of seeding the soil with such organisms was raised. The experiments which have been conducted on seeding land with soil in which the specific organisms of any leguminous tubercles are known to exist, have sometimes given positive and at others only indifferent results.

The positive results have usually come from the inoculation of virgin soils, moorlands or barren soils that have not previously born leguminous crops.

Salfeld,¹⁰⁰ in North Germany, obtained excellent results on the large scale on recently reclaimed moorlands sown to peas and beans which had received applications of lime, phosphatic slag, kainit and nitrate of soda, together with small quantities of fertile loam from fields that had previously born good crops of these plants.

In this case it was probable that the soil was deficient in nodule producing bacteria. Freewith¹⁰¹ found that in plots containing lupines, and inoculated with lupine soil at a rate of 1,600 to 3,200 pounds of earth per acre, there was an increase in yield of stems, leaves and hulls of from 67 to 72 per cent. In plots containing serradella, and inoculated with serradella soil, at the rate of 80 pounds to the acre, there was an increase of yield of fodder of from 41 to 282 per cent.

In the latter case tubercles were found on the roots of the plants grown in inoculated soil, while they were absent in those grown on uninoculated land. In this case there must have been an absence or deficiency of the proper organisms present, which were supplied by the soil inoculations.

Soils which have been previously uncultivated are more likely to respond to soil inoculation than those which have been under tillage. Thus Schniftes¹⁰² found that when cultivated clay soil was inoculated with earth containing bacteria from the root tubercles of lupines no favorable results were obtained. But when soil previously uncultivated was inoculated in the same way the increase of yield was from 11 to 32 per cent.

Otis,¹⁰³ of Kansas states that soy beans have been grown for eight years at the Kansas Station, but during that time tubercles were not found on their roots. Accordingly he secured soil from Massachusetts in which tubercle bearing soy beans had been grown. This soil was used to inoculate the Kansas soy beans. All the inoculated plants showed tubercles; there was also, as compared with uninoculated plants, a greater diameter of the lower part of the stem. Analyses of the crop showed also a slight increase in nitrogen, protein and water content.

Instead of soils, use has recently been made of cultures of the bacteria found in the root tubercles. Such cultures have already become a commercial product under the name of *Nitragin*.

The organisms are grown upon a specially prepared gelatin medium. Cultures from different legumes are made and sold, so that the proper organisms can be supplied for any given leguminous plant.

In using the cultures, the tubes containing them are placed in luke-warm water having a temperature of about 90 degrees F., to melt the gelatin and disseminate the germs throughout the medium. This is then mixed with a small quantity of water, and in this the seed is immersed previous to sowing, or the diluted cultures can be mixed thoroughly with earth and the latter sown broadcast over the land, immediately after the seed is sown. In immersing the seed in the solution the germs are brought into immediate contact with the developing plant, when root infection is more apt to follow.

The experiments on the use of nitragin have been so extensive and varied that it will be unprofitable to detail them here. Furthermore, the results have been so contradictory that confusion is likely to overwhelm the reader were we to review them.

In many cases increased yields have followed the use of nitragin, in others no benefit has resulted. Perhaps the failure in many cases has been due to the use of inactive nitragin, but, in most instances, it was likely owing to the fact that the necessary organisms were already abundantly present in the soil.

The question of the inoculation of soils, either with soils or with nitragin, therefore, resolves itself into one of whether the necessary organisms are already present.

In the cases where beneficial results have been attained, the soils on which it was used was either barren or otherwise below the standard of what might be classed as fertile soils. In the case of poor, sandy or worn-out lands, nitragin will doubtless be useful in initiating a good growth of any particular legume, until by a first crop the soil shall become well supplied with the necessary bacteria; after that there would be no advantage in its further use. In ordinary arable land some form of the tubercle organism is probably present in proper numbers to infect the roots to a degree sufficient at least to stock the soil with organisms of the proper virulence for another season.

Cases where nitragin is advisable are, therefore, the exceptions rather than the rule, and on this point no hard and fast lines can be drawn. The indiscriminate use of nitragin has led to many failures and disappointments. Like all good things when wisely used it is useful, but when used without judgment it is liable to meet with unjust condemnation.

7. INCREASE OF THE NITROGEN CONTENT OF SOILS.

It is a familiar fact that land left to itself in an undisturbed condition increases in fertility from year to year. In the forests and prairies of our own country we have profited by the accumulated fertility of centuries, and much of our present National wealth we owe to those biological processes of the soil we are about to enumerate.

By these we mean the ability of the soil to accumulate nitrogen through the agency of microscopic organisms, and the lower classes of plants. Every observer is familiar with the growth of lichens on bare rocks on which no organic food, much less nitrogen in any appreciable quantity, is present; and yet such plants contain nitrogen and contribute it to the thin mantle of soil which slowly accumulates over rocky surfaces.

Fungi are frequently found on sterile sand in which only an inappreciable quantity of nitrogen is present, and yet they may store up in their tissues quantities of nitrogen greatly in excess of what the soil is able to furnish.

Mulder showed that moulds grown upon non-nitrogenous substances always contain protein, and his experiments are quoted by Storer.

In aqueous solutions of sugar left "for three months in stoppered bottles, with a seven fold volume of air, an abundance of mould grew, which, on being collected and subjected to dry distillation gave off large quantities of ammonia. So, too, starch kept under water in a bottle that contained air soon fermented, and the fungus which it had nourished gave off ammonia on being distilled."

It is now generally recognized that fungi, microscopic algae, and perhaps other of the lower cryptogams, possess greater or less power of fixing atmospheric nitrogen.

Bertholet¹⁰⁴ held, and, in fact demonstrated, that certain microscopic organisms of the soil do appropriate the free nitrogen of the air. During the growing season, in clayey and sandy soils, he observed a slow but continual fixation of nitrogen from the air. This increase, furthermore, failed to take place when the soils were sterilized by heating them to 230 degrees F. From this it was concluded that the fixation of nitrogen was due to certain micro-organisms which were destroyed by the heat. Bertholet, from his laboratory experiments, calculated that as much as 75 to 100 pounds of nitrogen per acre could be fixed in this way, and in two exceptional cases as high as 525 and 980 pounds to the acre.

A number of chemists, notably, Koenig, Kiesow, Armsby, Birner, Kellner, Dehérain and Avery¹⁰⁴ found that when organic matter in one form or another undergoes fermentative changes of a putrefactive character, there was frequently an increase of nitrogen in the

fermenting substance. This was particularly marked when the materials were kept alkaline with carbonate of soda or lime.

The explanation given for this increase was that a combination of nascent hydrogen evolved during the fermentation took place with the free nitrogen of the air by which ammonia (NH_3) was produced.

It is known that hydrogen is a common product of putrefactive fermentation, and it is highly probable that when such putrefactive changes take place in the soil there is a certain fixation of atmospheric nitrogen.

The claim of Bertholet that micro-organisms of the soil possess the power of utilizing free nitrogen led Winogradsky¹⁰⁵ to test the validity of this assertion. Of 15 separate species of soil bacteria isolated by him only one of them was able to assimilate nitrogen to any appreciable degree. To this he gave the name *Clostridium pasteurianum*.

Cultures of the latter were made in saccharine media free from combined nitrogen, in which the organism grew, apparently dependent upon the free nitrogen of the atmosphere for their food. The conclusion which the author reaches, however, is that the power of fixing nitrogen is not general among micro-organisms.

Since Winogradsky's investigations of the above, a few others have been isolated which are capable of fixing free nitrogen, notably, *Bacillus ellenbachiensis*, which is now sold in the form of cultures known as *alinit*.

The fact that but few species of bacteria possessing the power of utilizing free nitrogen have been found is no argument that they do not exist in the soil, and the fact that soils do make appreciable gains in nitrogen is indication that the necessary organisms must be present.

From a practical standpoint the matter has little significance, since the gains of soils from this source are in a single year inconsiderable as compared with those which can be so readily brought about by the growth of legumes.

To effect results of momentous importance their action must cover long periods of years such as have passed before the advent of European agriculture on the American continent.

8. THE USE OF ALINIT.

All great discoveries are likely to lead to spasmodic efforts to revolutionize agriculture, and the climax has been reached in the effort to seed on soils with nitrogen assimilating bacteria by the use of alinit.

Alinit is a culture of *Bacillus ellenbachiensis*, which organism

is claimed to possess the power of utilizing atmospheric nitrogen. It is supposed to be particularly valuable in the growth of the cereals which lack the power of utilizing free nitrogen.

In the use of alinit, both beneficial and indifferent results have been obtained, but the experience in its use have not been as yet extensive enough to warrant definite conclusions being drawn regarding its value; but from a theoretical standpoint there is little to recommend it.

B. ellenbachiensis is probably identical with *B. megatherium* which is commonly present in soils, in which case nothing new is added to the soil by its use. Furthermore, of the myriads of bacteria present in a handful of soil, the addition of a relatively infinitesimal quantity of alinit would be like a drop of water added to a reservoir, and in the struggle for life between these myriads of soil bacteria there is a question whether one *B. ellenbachiensis* in a million other forms would be certain of a maintained existence, or at any rate be liable to multiply to a degree in excess of the others, sufficient to produce appreciable results.

If soil bacteria in general, as is probably the case, have any important relation to nitrogen assimilation, more is to be gained in following those methods already set forth, which aim to provide for the best development of soil bacteria in general.

At best, the gain of nitrogen from the use of artificial cultures, or from the stimulation of the development of any nitrogen assimilating bacteria already present, will be far below the demands of agricultural plants, and also inferior to the means which we have in the utilization of leguminous plants in our systems of rotation.



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